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A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

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Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding*

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Statistical analyses (9, 17) have established a relation between body weight and the development of tumors, and experimental studies have indicated that caloric restriction alone is sufficient to reduce the incidence of some neoplastic diseases and to delay their occurrence (11, 18, 19). The present experiments add another example to this established relationship and furnish some information on the mechanism of the inhibition of tumor growth by caloric restriction.

Tannenbaum (18) found that limitation of the body weight of mice by caloric restriction resulted in a lowered frequency of spontaneous mammary tumors and of induced skin tumors and sarcomas. McCay and his associates (11) retarded the growth of rats by drastic quantitative restriction of a diet adequate in essential food components. Spontaneous tumors of various types were less frequent in these rats than in normally fed controls at corresponding ages. Analysis of life insurance statistics made by Tannenbaum (17) has suggested that cancers develop more often in overweight persons than in those of normal or subnormal weight. Loeb and his associates (9) concluded that the tendency to develop mammary carcinoma increases with body weight. Visscher and his group (19) found that restriction of caloric intake inhibits the development of spontaneous mammary carcinoma in mice of the C3H strain. Studies on the relations of various amino acids and other constituents of the diet to tumor formation have been reviewed elsewhere (6). The evidence presented by Tannenbaum and others indicates that apart from the influence of specific substances of the diet on tumor growth there exists a direct relation between energy content of the diet and tumor growth. It was therefore of interest to determine the effect of underfeeding upon the development of leukemia in a strain of mice having a known high frequency of this disease.

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MATERIALS AND METHODS

The mice used were of the Ak stock, inbred for over 25 generations by brother and sister matings. The incidence of lymphoid leukemia in this stock is nearly 70 per cent in mice living past the age of 7 months (1); the incidence of the disease in different sublines varies from approximately 55 to 75 per cent. When infections, particularly pneumonia, are prevalent the figures are low, but have not been less than 55 per cent. Engelbreth-Holm (3) found the incidence of leukemia in this stock to be 50 to 70 per cent.

From the time of weaning, one group of 47 male and 47 female mice was fed the normal colony diet in an amount sufficient to permit only the minimum of growth and gain in weight compatible with life. This diet consisted of pellets of Tioga dog food, and bread and milk supplemented with cod liver oil and dried yeast; in both cases a weekly feeding of carrots was given. The amount fed was determined according to the daily or weekly weights of the mice and adjusted from time to time, depending upon the condition of the animals. A mouse could be maintained on about 2 gm. of bread and milk and 1.5 gm. of the dog food pellets given on alternate days. During the course of the experiment the diet was changed to Wayne "Fox Food Blox," 1.5 gm. being fed daily to each animal. Water was supplied at all times. After the first 6 months of the experiment, the mice were caged individually.

A second group, consisting of 52 male and 59 female mice, served as controls. These mice were given the same diet but in unlimited amounts. They were not caged individually but were usually segregated according to sex.

With the exceptions noted, both groups, underfed and control, were kept under as uniform conditions as possible until natural death occurred. All dead animals were autopsied; microscopic examinations were made only when the diagnosis was not clear. In most instances the body length was measured. During the course of the experiment, all animals were weighed weekly.

The 7 underfed mice killed at 9 to 17 months of age for the bioassays that will be described below are included in the charts and in the tabulations of life span and frequency of leukemia, those with positive bioassays as leukemic, the others as negative. Exclusion of these 7 animals from the group would bring the figures for the incidence of leukemia among the underfed animals down to 6.9 per cent instead of the 10.1 per cent given.

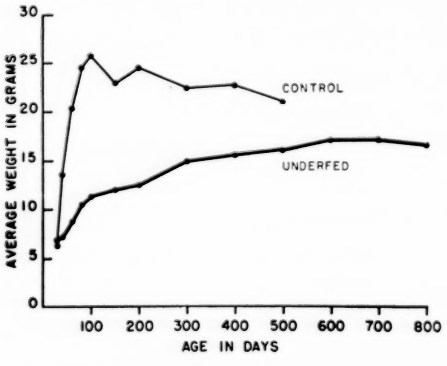

FIG. 1.—Average weights of control and underfed mice from weaning (4 weeks old). Figures for first 100 days are average weights of 7 underfed and 7 control mice. Figures for 150 to 800 days are average weights of a representative group of surviving animals in the experiment.

The 21 normal mice killed for the bioassays at 6 to 11 months of age are not included in the charts or in the tabulations.

GROWTH AND DEVELOPMENT OF UNDERFED MICE

In Fig. 1 are curves of the average weights of the underfed and control mice. At 400 days of age the underfed mice weighed about two-thirds as much as the controls. At later ages the underfed animals were of about the same weight as normal old mice of this stock. The average body length (measured from nose to anus) of the underfed mice was also slightly less than that of the controls, the values being 8.5 and 9.5 cm. respectively. The caloric restriction inhibited reproduction; during the first 6 months of the experiment the underfed mice were grouped in cages without regard to sex, and none of the females became pregnant. The vagina remained closed for as long as one year, and at autopsy the uterus was invariably thin and the ovaries appeared inactive. In males the seminal vesicles remained small. No instance of fighting, evidence of sexual maturity, occurred among the underfed male mice. In contrast, fighting was common among the control males, and litters were born in one cage of control mice of mixed sexes. To eliminate the possible effect of reproduction upon the frequency of leukemia the controls, except for those in one cage, were segregated according to sex.

The underfed mice appeared physiologically younger than the controls of corresponding age. Not only were they smaller, but their fur remained thick and fine in texture at an age when that of normal mice appeared sparser and coarser.

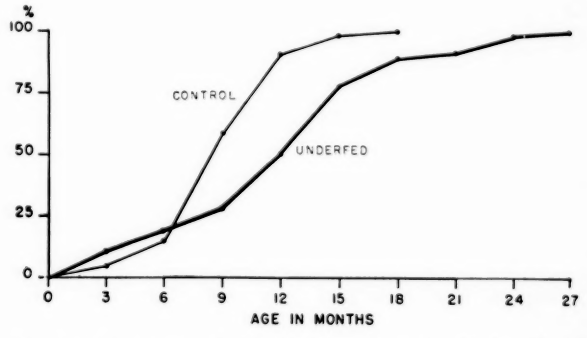

FIG. 2.—Percentage of animals of each group dead at the end of each 3 month period.

LIFE SPAN AND FREQUENCY OF LEUKEMIA IN UNDERFED MICE

Mice dying at less than 6 months of age are omitted from the tabulations of life span and frequency of leukemia, but the charts include all animals. A number of early deaths occurred among the controls as a result of fighting and of pneumonia. The cause of early death of the underfed mice was usually not evident at autopsy and was attributed to the drastic restriction of diet. A total of 11 control and 15 underfed mice are omitted, constituting 10 and 16 per cent respectively of the original group. It was thought that these early deaths did not introduce a serious selective effect upon the frequency of leukemia in the two groups.

In Fig. 2 are shown curves of mortality of the control and underfed groups. Although there was a slightly higher mortality among the underfed mice in the early months of the experiment, members of this group that survived until the end of 6 months lived on the average longer than the controls that were alive at this age. About 90 per cent of the controls

were dead at the end of 12 months, whereas only 50 per cent of the underfed mice had died. No control animal lived longer than 18 months; but several underfed mice lived for longer periods, one reaching the age of 27 months. This mortality curve is similar to that obtained by McCay and his associates for rats retarded in development by similar dietary restriction (12). It is thus apparent that underfeeding prolonged both the average and the maximum life span. The average life spans of underfed and control mice living beyond 6 months of age were 14.0 and 9.6 months respectively.

Underfeeding did not influence the anatomic manifestations of leukemia. In both underfed and control mice the disease was characterized by great enlargement of lymph nodes and spleen, frequently with a tumor-like mass involving the thymus.

The average age at death in animals dying of causes other than leukemia was greater in the underfed than in the control group, the values being 14.0 and 10.5 months respectively. This indicates that underfeeding influenced the average life span of mice of this strain by its effect upon other diseases as well as upon spontaneous leukemia.

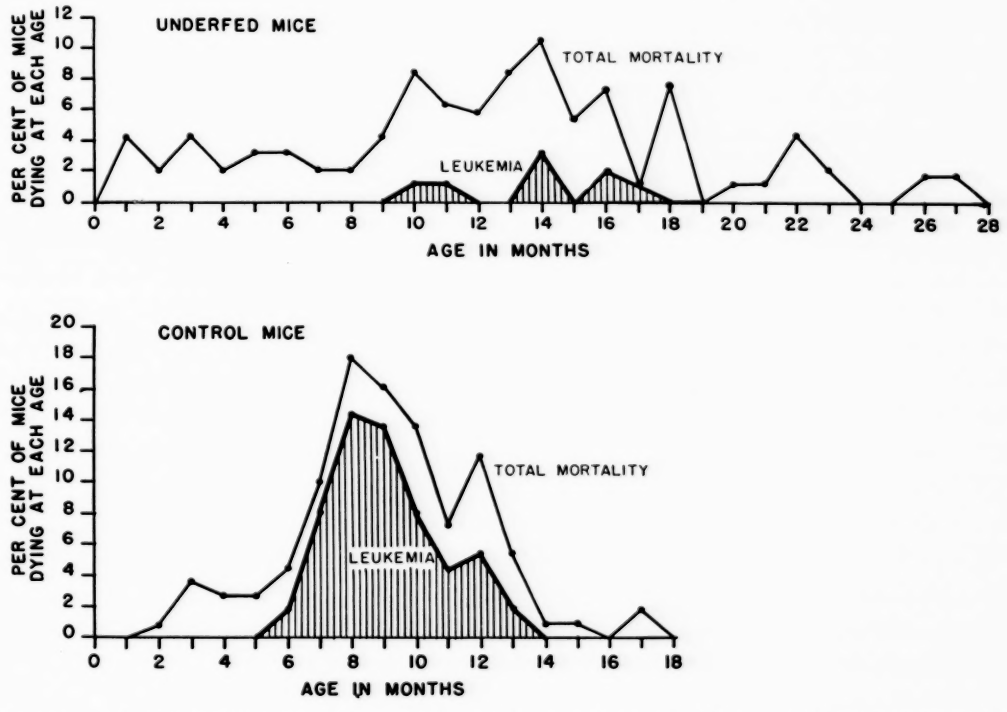

FIG. 3.—Percentage of animals dying at each month of age. Lines enclosing shaded areas indicate percentage of animals dying with leukemia.

The frequency of leukemia in the control and underfed groups at comparable ages is shown graphically in Fig. 3. Leukemia occurred at an earlier age and with greater frequency in the controls than in the underfed mice, the frequencies being 65.0 and 10.1 per cent respectively. It might be justifiable to exclude from the total number of underfed mice the 7 animals that were killed for bioassays. If this procedure is adopted, the figure for the incidence of leukemia among the underfed animals would be 6.9 per cent. The larger number of normal mice killed for bioassays at a somewhat younger age was not part of the original control group and has not been included in the calculation of the data. The youngest animals to develop leukemia were 2 female controls, dying at the age of 6 months; the youngest leukemic animal in the underfed group was 10 months of age at death.

Bioassays for Leukemic Cells in the Tissues of Normal and Underfed Animals

What is the mechanism of retardation or prevention of leukemia by underfeeding? As underfeeding prolongs the life of mice with transmitted leukemia (5) it might be supposed that the malignant lymphocytes remain dormant or proliferate slowly in underfed animals. It is also possible, however, that the malignant transformation may be prevented by underfeeding. To test these possibilities normal and underfed animals were killed at various ages, and cell suspensions from organs that are the usual sites of the malignant lymphoid cells were introduced into young mice highly susceptible to leukemia. The results of these tests, termed bioassays for leukemia, are presented in Tables I and II.

The recipient mice were of the high-leukemia stock Ak or their first generation hybrids, every member of which is receptive to leukemic cells of this stock. They were killed 87 to 130 days after injection, some of them at a time when they had reached the age at which they may have had spontaneous leukemia. For this reason, unless the recipient mice were younger than 5 months of age at time of death the possibility that leukemia was spontaneous will be given special consideration. Leukemia produced by transmission usually killed the mice within this period.

The mice used as donors for the tissues to be bioassayed were anesthetized with ether, the heart was exposed, and the blood removed from the heart with a heparin-rinsed syringe and 26-gauge needle. One-tenth cubic centimeter of the heart blood was injected immediately into the tail vein of the recipient mice. In preparing cell suspensions from spleen, thymus, lymph nodes, and bone marrow the organs were minced in approximately 0.5 cc. of Tyrode's solution. The cell suspensions were filtered through cotton and 0.1 to 0.2 cc. injected intravenously into each of the recipient mice. Except for a small piece reserved for sectioning, the entire spleen and thymus were used for inoculation. From the underfed mice, the entire thymus together with the mediastinal lymph nodes was used. The superficial, retroperitoneal, and mesenteric lymph nodes were pooled, except for one node reserved for sectioning, in the preparation of cell suspension. In preparing the bone marrow suspensions, both humeri and one femur were freed from muscle and connective tissue, crushed in Tyrode's solution, and a cell suspension was prepared in the usual manner. The other femur was fixed for microscopic study.

The results of the bioassays, summarized in Table I, indicate that in normally fed animals 6 or 7 months old none of the organs tested (blood, lymph nodes, thymus, spleen) harbored malignant cells. When tested at 8 to 11 months, approximately one-half of the normal mice harbored malignant lymphoid cells, as indicated by this procedure, even though in the majority of instances neither gross nor routine microscopic examination suggested that the donor mice had leukemia.

These figures do not faithfully represent the time of onset of leukemia in the Ak stock, since with two exceptions bioassays were not made on the tissues of animals selected for this purpose whenever the gross postmortem examination indicated leukemia. The first exception was a mouse (Ako 41) killed at 8 months of age that had a mediastinal lymphosarcoma, and the bioassays were performed to find out whether other organs harbored malignant cells, although they were free from leukemic infiltration in the gross. The spleen contained them in large numbers, but the blood did not (Table I). Another mouse (Akh 1620), killed at 9 months of age, had a mediastinal lymphosarcoma, but generalized leukemia was not clearly recognized at autopsy. Lymph nodes, spleen, and thymus all contained malignant cells, as indicated by the bioassay findings.

The results of the bioassays on the tissues of normal animals indicate that both gross and microscopic examinations are crude procedures for the disclosure of malignant cells. Obviously, then, when the anatomical examinations indicate leukemia, however scant the changes, the disease has passed its earliest phase.

In the bioassays with the tissues from mouse Akl 434 only 1 test mouse died with leukemia, and this occurred 6 months after the injection; this recipient mouse may have developed spontaneous leukemia. Inoculations with tissue from mouse Ak 1754 yielded similar results. One mouse that received a bone marrow suspension from this animal developed mediastinal lymphomatosis, an unlikely event following intravenous transmission of either lymphoid or myeloid leukemia. In bioassays from Ak 1752 one test animal injected with thymic cells died with leukemia 87 days after injection, but all other "takes" were noted at autopsy performed 110 days after injection, when these mice were 6 months old. At this age an occasional Ak mouse dies of leukemia, but it is most unlikely that so many animals of a small group should have the disease at this age. It seems, therefore, that the thymus, lymph nodes, spleen, and blood of this mouse contained malignant lymphocytes. Failure to recover leukemic blood cells from the bone marrow of this mouse is noteworthy.

Attempts to correlate morphological blood changes with transmissibility of the disease by injection of blood have been unsuccessful. Leukocyte counts were not made but the total number of leukocytes, as roughly estimated from blood smears, appeared to be within the normal range and differential counts failed to indicate leukemia, yet the blood from 3 mice (Ak 1752, Ak 1739, Ak 1750) when injected into young susceptible animals was capable of producing leukemia. The total percentages of lymphocytes in these 3 mice were 64.5, 58, and 44.5, and the percentages of basophilic lymphocytes of medium and large size 5.5, 4.5, and 3.5. The latter figures are not unusual among normal mice in our experience. The highest percentages of lymphocytes were from 2 mice that were killed at 8 months of age (Akl 466, Ako 41), whose blood failed to transmit the disease

TABLE I: RÉSUMÉ OF BLOOD COUNTS, ANATOMICAL FINDINGS, AND BIOASSAYS IN NORMAL AK MICE

Donor	Age in mos.	Differential white blood cell count (%)			Anatomical findings	Bioassay of:				
		Granulocytes	Lymphocytes	Basoph. lymphocytes		Blood	Lymph nodes	Thymus	Spleen	Bone marrow
Akl 497	6	40	53	2.5	No leukemia	0/5* K122†, 6 mos.‡	0/5 K122, 6 mos.	0/5 K122, 6 mos.	0/5 K122, 6 mos.	—
Akl 485	6	32	66.5	3	No leukemia	0/5 K124, 7 mos.	0/5 K124, 7 mos.	0/5 K124, 7 mos.	0/4 K124, 7 mos.	—
Akl 464	6	48.5	49	4	No leukemia	0/5 K129, 6 mos.	—	0/4 K129, 6 mos.	0/5 K129, 6 mos.	—
Akl 465	7	65.5	32.5	1.5	No leukemia	0/5 K130, 6 mos.	0/4 K130, 6 mos.	0/5 K130, 6 mos.	0/4 K130, 6 mos.	—
Akn 58	7	29.5	65.5	4	No leukemia	0/4 K128, 7 mos.	0/5 K128, 7 mos.	0/5 K128, 7 mos.	0/4 K128, 7 mos.	—
Akl 466	8	25.5	70.5	2	No leukemia	0/5 K122, 6 mos.	0/5 K122, 6 mos.	0/5 K122, 6 mos.	0/5 K122, 6 mos.	—
Akl 434	8	33.5	64	2.5	No leukemia	0/5 K128, 7 mos.	0/4 K128, 7 mos.	0/5 K128, 7 mos.	1/5 D or K109-128, 6-7 mos.	—
Ako 41	8	20.5	72.5	3	Mediast. lymphosarcoma	0/5 K129, 6 mos.	—	2/2 D27-30, 2-3 mos.	5/5 D41-44, 3 mos.	—
Akh 1620	9	—	—	—	Gener. lymph. leukemia	—	6/6 D26-74, 3-5 mos.	5/5 D16-37, 3-4 mos.	4/4 D20-53, 2-4 mos.	—
Akl 396	9	—	—	—	No leukemia	—	0/5 K125, 5-6 mos.	0/5 K125, 5-6 mos.	0/5 K125, 5-6 mos.	—
Akl 399	9	—	—	—	Hyperplasia; ± for lymph. leukemia	—	5/5 D60-66, 3-4 mos.	3/3 D24-53, 2-3 mos.	5/5 D51-88, 3-4 mos.	—
Ak 1731	9	32.5	57.5	5	No leukemia	0/4 K98, 5 mos.	0/3 K98, 4-5 mos.	0/3 K98, 4-5 mos.	0/4 K98, 4-5 mos.	0/4 K98, 4-5 mos.
Ak 1752	9	52	44.5	3.5	No leukemia	1/4 K110, 6 mos.	2/4 K110, 6 mos.	2/4 D or K87-110, 5-6 mos.	1/4 K110, 6 mos.	0/4 K110, 6 mos.
Akl 411	10	49.5	46	5	No leukemia	0/5 K122, 6 mos.	0/5 K122, 6 mos.	0/4 K122, 6 mos.	0/4 K122, 6 mos.	—
Akl 413	10	65	30	0	No leukemia	0/5 K123, 6 mos.	0/5 K123-127, 6 mos.	0/4 D or K104-127, 6 mos.	0/5 K123-127, 6 mos.	—
Ak 1739	10	27.5	64.5	5.5	No leukemia	3/3 D or K80-108, 5-6 mos.	1/3 K107-108, 6 mos.	3/3 D or K92-102, 5-6 mos.	3/3 D or K82-120, 4-6 mos.	1/2 K108, 5 mos.
Ak 1744	10	35	59	3	No leukemia	0/4 K107, 5 mos.	0/4 K107, 5 mos.	0/3 K107, 5 mos.	0/4 K107, 5-6 mos.	0/3 K107, 5-6 mos.
Ak 1754	10	41	57.5	4	No leukemia	0/4 K90, 4-5 mos.	0/4 K90, 4-5 mos.	0/4 K90, 4-5 mos.	0/4 D or K76-90, 4-5 mos.	1/4 K123, 6 mos.
Ak 1748	10	22	65.5	8	Lymphoid hyperplasia	0/4 K93, 4 mos.	1/3 K93, 4 mos.	1/4 D or K88-105, 4-5 mos.	1/4 D or K82-93, 4 mos.	0/4 K93, 4 mos.
Ak 1750	11	39	58	4.5	Lymphoid hyperplasia	1/4 K87, 4 mos.	1/4 K87, 4 mos.	0/4 K99, 5 mos.	0/4 K87, 4 mos.	0/4 K99, 4-5 mos.
Ak 1753	11	41	55.5	4	Lymphoid hyperplasia	0/4 K95, 4 mos.	1/3 D or K91-95, 4 mos.	1/4 D or K76-95, 4 mos.	0/4 K95, 4 mos.	1/4 K95, 4 mos.

* Number of injections yielding leukemia over number of mice injected.
† K = killed; D = died, after the given number of days following injection.
‡ Age in months of recipient mice at termination of experiment.

even though spleen and thymus suspensions from one (Ako 41) produced leukemias. The percentage of basophilic lymphocytes in these animals was within the normal range. Thus at the early stage of leukemia in mice diagnosis could not be made from blood smears. The results of the bioassays indicate that a certain number of mice whose blood smears do not suggest leukemia harbor malignant lymphocytes in the blood. The negative bioassays are likewise of interest. Earlier experiments have shown that all spontaneous lymphoid leukemias from the highly inbred Ak mice that were tested proved readily transmissible with small numbers of cells. There are, however, no precise data on the number of malignant lymphoid cells that are required to effect the first passage of a spontaneous leukemia. Failure to transmit the disease with 0.1 cc. of blood, that is, by the injection of approximately 1,000,000 circulating leukocytes, strongly suggests, therefore, that leukemic lymphocytes do not circulate in the blood at all times during the early stage of illness. Earlier experiments have shown that malignant lymphocytes introduced intravenously into susceptible normal mice disappeared from the blood, proliferated in tissues, and reappeared in the blood at the late stage of the disease (8).

All 3 mice injected with blood of mouse Ak 1739 (Table I) died of leukemia, even though the total number of circulating leukocytes of this mouse and the differential count appeared normal. The malignant cells of this animal had a special ability to invade the blood, as indicated by the tremendous number of leukocytes seen in blood vessels in sections from a successfully inoculated animal that died at an advanced stage of the disease.

The results of those bioassays from *normally fed mice* that yielded leukemia presumably resulting from transmission, are surveyed in the tabulation that follows:

Thus the lymph nodes were the only organs from which the inoculations were successful in every case; they also yielded the greatest percentage of successful inoculations. Next in order, if not equal to lymph nodes, were thymus and spleen. The bone marrow and blood, however, only occasionally harbored malignant cells at the earliest phase of leukemia. Indeed, the successful inoculations with bone marrow can be accounted for, at least in some instances, by its blood content. The type of leukemia encountered in this series was almost exclusively lymphoid. In both spontaneous and transmitted lymphoid leukemia the bone marrow may be free from leukemic infiltration or involved only at an advanced stage of the disease.

The results of *bioassays on the tissues of underfed animals* are presented in Table II. All tissue suspensions from the 4 underfed mice killed at 9 and 12 months of age failed to produce leukemia. By contrast, leukemia was revealed by the bioassays in mice of the control group killed at 8 months. All 3 underfed mice, killed at 14, 16, and 17 months respectively harbored malignant cells, however, as indicated by the bioassays. These 3 mice appeared normal on gross and microscopic examinations, and the antemortem differential count from one of them was within the normal range.

A tabulation of successful transmissions from tissues of underfed animals is shown in the unnumbered table on page 407.

The results are comparable with those obtained in bioassays with tissues of normally fed animals. The highest percentage of successful inoculations was obtained with cell suspensions from lymph nodes. The seemingly normal blood of an underfed animal carried malignant cells, while its bone marrow used for inoculations did not. The combined figures from normal and underfed animals indicating the percentage of successful inoculations were: lymph nodes 64 per cent, spleen 59 per cent, thymus 57 per cent, blood 26 per cent, and bone marrow 9 per cent.

	Source of cell suspension from normal animals									
	Blood, No. of mice		Lymph nodes, No. of mice		Thymus, No. of mice		Spleen, No. of mice		Bone marrow, No. of mice	
Number of donor	Inj.	+	Inj.	+	Inj.	+	Inj.	+	Inj.	+
Ako 41	5	0			2	2	5	5		
Akh 1620			6	6	5	5	4	4		
Akl 399			5	5	3	3	5	5		
Ak 1752	4	1	4	2	4	2	4	1	4	0
Ak 1739	3	3	3	1	3	3	3	3	2	1
Ak 1748	4	0	3	1	4	1	4	1	4	0
Ak 1750	4	1	4	1	4	0	4	0	4	0
Ak 1753	4	0	3	1	4	1	4	0	4	1
Total	24	5	28	17	29	17	33	19	18	2
Takes		21%		61%		59%		58%		11%

COMMENTS

Earlier experiments have shown that underfeeding prevents or delays the occurrence of tumors of different sorts (11, 18, 19), and the present experiments indicate that it has the same effect on spontaneous leukemia.

The question arises how this effect is brought about. It may be due to a lower energy content of the diet, or to deficiencies of certain dietary constituents. It seems unlikely that animals deficient in some essential substance should outlive those receiving it and the carefully controlled experiments of Tannenbaum (18), McCay (11), and Visscher and his associates (19) support the assumption that the effect of underfeeding in reducing the incidence of tumors is due to limitation of energy content alone.

but it never prevented the fatal termination of the disease. These experiments with transmitted leukemia indicate that the proliferation of malignant cells is retarded by underfeeding.

To learn if the frequency of malignant transformation is also influenced by this procedure, bioassays were made on different tissues of normal and underfed mice at various ages by injecting cell suspensions into young mice highly susceptible to leukemia. It was hoped that these bioassays would disclose where and when malignant cells appear in organs of mice of the high-leukemia stock. The results indicate that in normal animals of this stock a malignant transformation of lymphoid cells takes place at about 7 to 8 months of age and that this is greatly delayed, but not entirely prevented, by underfeeding. Dietary control was not rigidly enforced after about 17 months of age, but the bioassays indicate that a malignant transformation had taken place in the underfed animals before this time.

Whether animals with a high hereditary susceptibility to certain neoplasms possess latent neoplastic cells at birth or at an early age, or whether a malignant transformation of normal cells occurs at a later age has not been satisfactorily answered by earlier investigations. Fischer (4) made successive transfers

TABLE II: RÉSUMÉ OF BLOOD COUNTS AND BIOASSAYS IN UNDERFED MICE

Donor	Age in mos.	Differential white blood cell count (%)			Bioassay of:				
		Granulocytes	Lymphocytes	Basoph. lymphocytes	Blood	Lymph nodes	Thymus	Spleen	Bone marrow
uAkh 1599	9	—	—	—		0/5 * K126 †	0/3 K126	0/5 K126	
uAkh 1585	9	—	—	—		0/5 K126	0/3 K126	0/5 K126	
uAkl 249	12	39	59	4	0/4 K120	0/5 K120	0/3 K120	0/5 K120	
uAkl 243	12	—	—	—		0/5 K125	0/3 K125	0/5 K125	
uAkl 211	14	—	—	—		5/5 D99-107	3/3 D57-119	4/4 D99-119	
uAkh 1587	16	52	41.5	2.5	2/3 K100	2/3 D or K 96-100	1/2 D or K 98-100	1/2 D or K 98-100	0/4 K100
uAkh 1503	17	—	—	—		2/5 D or K 107-125	0/3 K125	0/4 K125	

None of the underfed mice bioassayed had anatomical changes indicative of leukemia. Mice uAkl 211 and uAkh 1503 had lymphoid hyperplasia.

* Number of injections yielding leukemia over number of mice injected.

† K = killed; D = died, after the given number of days following injection. All recipient mice were approximately 5 months of age at termination of experiment.

Source of cell suspensions from underfed animals

Number of donor	Blood, No. of mice		Lymph nodes, No. of mice		Thymus, No. of mice		Spleen, No. of mice		Bone marrow, No. of mice	
	Inj.	+	Inj.	+	Inj.	+	Inj.	+	Inj.	+
uAkl 211			5	5	3	3	4	4		
uAkh 1587	3	2	3	2	2	1	2	1	4	0
uAkh 1503			5	2	3	0	4	0		
Total	3	2	13	9	8	4	10	5	4	0
Takes		67%		69%		50%		50%		0%

An inhibition of the development of transmitted leukemia occurred (5) when the host mice were underfed in the manner here described, following inoculation with cells of several transmissible strains of leukemia. The transmitted leukemia usually killed the hosts in less than 4 weeks, and it is doubtful that serious specific deficiencies could occur in so short a time by mere reduction of the quantity of a well-balanced diet. Underfeeding prolonged the life of mice with transmitted leukemia by about 20 per cent,

of mammary tissue in mice, arriving at the conclusion that this procedure is suitable for demonstrating the presence of dormant malignant cells in the breast at an early age. However, the neoplastic potentialities of the mice he used were not ascertained precisely, and the causation of mammary cancer in mice by a transmissible milk factor complicates the evaluation of Fischer's work. The recent tissue culture studies of Earle (2) suggest that with induced rapid multiplication of cells, such as occurs in cultures *in vitro,* malignant transformation may take place.

As concerns the sites of malignant transformation, bioassays thus far have yielded only fragmentary data. When the inoculations were successful, "takes" were usually scored with several organs, namely, lymph nodes, spleen, and thymus. It follows that if leukemia is unicentric in origin the malignant cells soon find their way into the circulation and spread to and proliferate in numerous organs. Bioassays of seemingly normal blood made at the early stage of the evolution of leukemia indicate that the blood may carry malignant cells even when blood counts and differential counts are normal. However, the present experiments do not exclude the possibility that leukemia is multicentric in origin. It is highly probable that, as with other neoplasms, multicentric as well as unicentric malignant transformation may occur in lymphoid tissues and that either may terminate in generalized leukemia.

A special morphological study of the preleukemic and latent leukemic state was not made, but microscopic studies of tissues from animals that were bioassayed failed to disclose the earliest phase of leukemia. The morphological studies of Potter, Victor, and Ward (14) are concerned with the time of onset of leukemia and the possible source of the leukemic cells. They noted in the preleukemic period the presence of a reticular hyperplasia in lymphoid tissues, while the germinal centers appeared normal. These investigations suggested that the leukemic cells are derived from proliferating reticular cells. The value of their work would doubtless have been enhanced by bioassays.

With respect to the mode of action of underfeeding, yet another possibility has to be considered. Recent studies (13) indicate that lymphomas are much less frequent in Ak mice from which the thymus has been removed at an early age than in control animals of the same stock. The incidence of spontaneous leukemia in this experiment decreased from 77 per cent to 8 per cent in female and from 61 to 11 per cent in male mice. The lymphoid tissues in underfed rats are inhibited in development (15), and the thymus undergoes more rapid involution than in normal rats (16). In the present experiment a similar underdevelopment of thymus, spleen, and lymphoid tissues of underfed mice was noted. The possibility that the lowering of the incidence of leukemia by underfeeding is referable, at least in part, to hastened involution of the thymus requires further study.

SUMMARY AND CONCLUSION

The incidence of spontaneous lymphoid leukemia in a high leukemia strain of mice was 65.0 per cent in 100 normally fed control mice. In 79 related mice that received limited amounts of an otherwise adequate diet the incidence was 10.1 per cent. The length of life of these mice was considerably prolonged by underfeeding.

Bioassays at 9 and 12 months indicated the absence of malignant lymphocytes in the underfed mice, and disclosed their presence at 14, 16, and 17 months. In normally fed mice of the same strain malignant cells appeared at 7 to 13 months.

These experiments indicate that (a) malignant lymphoid cells are absent in young normal mice of the high-leukemia stock Ak; (b) the malignant transformation takes place shortly before leukemia becomes manifest; and (c) this transformation is delayed but not entirely prevented by underfeeding.

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Incidence of Spontaneous Fibroadenoma in the Albany Strain of Rats*

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In a previous communication (5) the origin of the Albany strain of rats, a strain characterized by an unusually high incidence of spontaneous benign mammary tumors in the females, was first described. Of the original group of 51 females of this strain, 26 developed spontaneous mammary fibroadenomas while 25 did not. By simple ratio, which is a method widely used by geneticists, the tumor incidence for this small group was therefore 50.9 per cent.

This incidence appeared to be very high for tumors of this type and for this species, since observations of previous investigators, notably Curtis, Bullock, and Dunning (6), and Heiman and Krehbiel (9), had indicated that the usual incidence of benign mammary tumors in large rat colonies was less than 1 per cent. This small group of rats, therefore, seemed to be the potential source of a strain that would yield a consistently high rate of spontaneous mammary tumors. It was considered that such a strain might prove to be valuable in studying the genesis of benign neoplasms of the breast.

In addition to the high incidence of spontaneous mammary tumors, these animals also showed a high degree of sterility (12). This characteristic, affecting most females to a variable degree, was found to be due to partial or complete failure of ovulation, which resulted in abnormalities of the estrous cycle. The ovarian dysfunction was severe enough to prevent approximately 30 per cent of the females from becoming pregnant. Moreover, even if pregnancy occurs in these rats, it does not always continue to term because of fetal resorption and excessive vaginal hemorrhage. In the males variable degrees of testicular degeneration have been observed, and this condition is undoubtedly responsible in part for the lack of fertility of the strain as a whole. There are evidences that these reproductive abnormalities are the result of an inherent dysfunction of the anterior lobe of the pituitary gland (13).

Nevertheless, in spite of the poor fertility, the present Albany colony has been established by inbreeding these animals. The inbreeding has been carried out mainly by brother-to-sister matings among the offspring of 2 of the original group of 51 females and their progeny. Thirteen substrains or lines were developed in this way, each line having been started by a single female and a single male. Of these substrains only 4 have yielded a sufficient number of tumor bearers to make it worth while to continue them. The remaining 9 either died out as a result of sterility or were discarded. Of the 4 lines that are still being continued one substrain, which will be referred to as Line 147, is showing a consistently increasing rate of tumor development.

Up to the present time we have assembled data concerning more than 4,600 animals that have survived to the age of 5 months, the earliest age at which mammary tumors have appeared. It seemed pertinent, therefore, at this time to analyze the data, in order to determine whether or not any real progress was being made in the development of a high-tumor strain of rats.

Methods of Measuring Tumor Incidence

It has long been apparent to investigators in the field of cancer research that the prevalent method of computing the inherent tendency to tumor development, that is, by simple ratio, is inadequate. Murray and Hoffman (11), recognizing the need of clarifying this problem, described and compared various methods available for computing the amount of cancer in a stock of animals. Previous to their publication, however, the method of obtaining a single index by the use of a standard population was also suggested to us independently by Dr. A. J. Lotka, of the Metropolitan Life Insurance Company, whose help it is a pleasure to acknowledge in this report. Although the methods used by us are amply described by Murray and Hoffman, the various calculations will be explained here for purposes of simplicity.

Tumor Incidence in the Albany Colony

A simple method of calculating tumor incidence is computation of the percentage of tumor-bearing animals that live to the beginning of each age period. This method, as Murray and Hoffman pointed out,

* These studies were aided by a grant from The International Cancer Research Foundation.

is superior to the method of simple ratio in giving information about the relation of the frequency of tumor development with advancing age. The procedure is illustrated in Table I. If in column 3 of Table I the numbers of new tumor bearers found each month are added from the specific age under consideration down through the oldest age at the bottom of the column, there is obtained a figure that represents the *total* number of rats that, among those surviving at the given age, were destined to develop tumors. For example, of the 1,140 rats alive at the age of 10 months (column 2), 503, the total of all the tumor-bearing rats that appeared at each age through the oldest, namely, 29 months, were destined, at the present rate of tumor development, to have a tumor. This total for each specific age is given in column 4. For any specific age, therefore, the percentage of tumor bearers among animals living to that age would be simply the ratio of the total given in column 4 to the number of animals surviving to that age, given in column 2. This percentage has been calculated and is given in column 5.

TABLE I: ILLUSTRATIVE TABLE SHOWING THE PERCENTAGE OF SPONTANEOUS FIBROADENOMAS AMONG RATS LIVING AT THE BEGINNING OF SUCCESSIVE CHRONOLOGICAL AGE PERIODS

	Line 147, October, 1943			
Age in months (1)	Survivors (2)	Rats developing new tumors (3)	Tumor bearers, total (4)	Tumor bearers, per cent (5)
5	1,477	0	518	35.0
6	1,396	1	518	37.1
7	1,319	0	517	39.2
8	1,257	5	517	41.1
9	1,198	9	512	42.8
10	1,140	31	503	44.1
11	1,079	26	472	43.8
12	1,018	53	446	43.8
13	916	46	393	42.9
14	819	44	347	42.4
15	716	29	303	42.3
16	607	51	274	45.1
17	508	40	223	44.0
18	405	36	183	45.2
19	330	23	147	44.6
20	284	18	124	43.7
21	244	27	106	43.5
22	190	13	79	41.5
23	145	17	66	45.5
24	108	23	49	45.3
25	69	12	26	37.7
26	48	6	14	29.2
27	28	3	8	28.5
28	15	2	5	33.3
29	11	3	3	27.2
30	7	0	0	0
31	4	0	0	0
32	3	0	0	0
33	3	0	0	0
34	3	0	0	0

The tumor incidence for the colony as a whole was calculated by this method in January, 1940; August, 1941; June, 1943; and October, 1943 (Table II). It will be noted that the tumor incidence has increased progressively during the period from January, 1940, to October, 1943. The incidence appears to be greatest between the ages of 12 and 18 months. After the 18th month the rates tend generally to fall somewhat. The figures for 1942-1943 are more reliable than those for 1940-1941, since they were obtained from greater numbers of survivors than were available in the earlier groups. When it is realized that only 1 per cent of the population reaches the age of 28 months, it can be appreciated that the percentages for the oldest groups are based on very small samples. Nevertheless, it is of considerable interest that beyond a certain degree of aging the frequency of tumor development tends actually to decrease.

The results indicate clearly that efforts to increase tumor incidence in the Albany colony have been successful. Of the 13 lines originally constituting the colony, one substrain, Line 147, has been showing progressively the greatest increase in tumor development. The tumor incidence for this line is also presented in Table II for 3 periods. The rate of appearance of mammary neoplasms is obviously so much greater for the calculations up to October, 1943, than for August, 1941, that this line seems to offer the best possibility for development of a high-tumor strain.

In analyzing the data it was of interest to compare the tumor incidence of breeding animals with that of rats that had never been pregnant. Many laboratories have reported that pregnancy results in an increase in the incidence of mammary carcinoma in mice (1, 2, 4, 10) and of fibroadenoma in rats (3). Reference to Table II will indicate that in this colony benign mammary tumors appeared more frequently in rats that had never been pregnant than in breeders. However, in Line 147, in which the samples are smaller, particularly for the breeders in the older age groups, the incidence among the nonpregnant animals was higher than for the breeders only up to about the 15th month.

The nonpregnant groups of rats included two types of animals, those that had been placed with fertile males and were found to be sterile, and virgins that had never been in contact with males. Although it is quite probable that many of the virgin rats were potentially fertile, the increased numbers of tumors in the sterile females certainly suggest the possibility that the endocrine imbalance responsible for the sterility was also associated in some way with the development of the fibroadenomas.

In order to obtain a single index by which the tumor incidence in the Albany colony or any specific

line, such as Line 147, could be directly compared with the rate of tumor growth in any other colony of rats showing the tendency to develop spontaneous fibroadenomas, it was necessary to use a calculation employing a standard population for reference. Inasmuch as there were not available to us any records of death rates of large colonies of rats outside the Albany colony, a standard population was derived from a composite death curve of nontumorous Albany rats that die from causes other than experimental.

Table II: Percentage of Spontaneous Fibroadenomas Among Rats Living at the Beginning of Successive Chronological Age Periods, as Illustrated by Table I

Age in months	Whole colony				Whole colony				Line 147			Line 147			
	Jan., '40	Aug., '41	June, '42	Oct., '43	Nonbreeders † 1942	Nonbreeders † 1943	Breeders 1942	Breeders 1943	Aug., '41	June, '42	Oct., '43	Nonbreeders † 1942	Nonbreeders † 1943	Breeders 1942	Breeders 1943
5 *	9.0	11.2	16.1	22.0	16.1	22.1	16.4	19.4	0	0	0	0	0	0	0
6	10.2	12.4	17.7	23.7	18.2	24.5	16.7	20.0	10.3	29.1	37.1	0	39.0	23.2	30.0
7	11.7	13.7	19.1	25.3	20.0	26.3	17.3	20.7	11.3	30.3	39.2	0	41.2	23.9	31.8
8	13.2	15.0	20.8	27.2	21.8	28.5	18.9	22.1	13.0	33.0	41.1	34.4	43.3	27.7	33.3
9	14.4	16.3	22.2	28.7	23.3	30.2	19.9	23.0	14.7	34.5	42.8	36.0	44.6	29.2	35.2
10	15.5	18.1	23.9	30.4	25.4	32.0	20.8	24.0	18.5	36.5	44.1	38.0	46.2	30.4	36.3
11	15.6	19.7	25.0	31.0	26.7	32.4	21.7	24.9	22.4	36.8	43.8	38.6	45.3	29.9	37.4
12	16.3	20.8	26.5	32.1	28.4	33.2	22.7	26.3	22.0	38.0	43.8	40.0	45.1	29.9	38.5
13	16.3	22.0	26.6	32.1	28.6	33.2	23.0	26.4	23.4	36.8	42.9	38.2	43.7	31.4	39.7
14	16.1	22.4	26.2	32.1	28.2	33.1	22.6	26.3	22.2	33.4	42.4	35.2	43.2	27.1	39.0
15	15.6	23.3	25.9	31.9	27.4	32.4	23.2	26.9	30.2	33.0	42.3	33.8	42.4	30.2	42.6
16	16.3	23.5	25.5	32.9	26.7	33.2	23.3	27.6	32.7	32.0	45.1	32.4	45.4	30.4	44.2
17	16.9	23.2	25.3	31.8	26.0	33.3	24.0	27.6	27.4	32.6	44.0	32.2	43.7	32.8	44.8
18	17.8	22.7	24.2	32.0	24.3	33.7	24.1	27.8	29.6	28.6	45.2	26.8	45.5	35.0	44.1
19	16.5	21.4	22.8	30.7	23.0	32.6	22.4	26.2	29.6	26.3	44.6	25.3	45.4	29.9	41.7
20	15.0	20.1	20.8	30.2	20.6	32.0	21.6	26.0	23.3	23.2	43.7	21.4	44.3	29.8	41.3
21	14.7	20.6	20.0	29.5	18.9	30.5	21.8	26.9	20.8	19.3	43.5	14.9	43.3	33.3	43.9
22	12.1	22.1	18.9	28.5	17.7	29.3	21.4	26.1	23.6	17.3	41.5	10.3	41.2	34.8	42.9
23	17.2	21.4	17.1	28.1	16.3	28.4	18.5	27.3	30.8	20.2	45.5	9.7	43.7	38.8	51.6
24	17.4	21.0	16.9	29.7	16.4	29.8	17.7	29.5	50.0	24.1	45.3	11.7	43.4	41.6	52.0
25	21.4	18.6	15.4	26.8	14.9	26.5	16.2	27.5	0	11.1	37.7	0	36.9	20.0	41.6
26	33.3	17.4	12.2	21.9	14.0	25.0	9.5	13.2	0	0	29.2	0	32.6	0	0
27	33.3	21.0	10.7	24.0	13.2	26.5	6.5	17.1	0	0	28.5	...	33.3	0	0
28	25.0	21.8	10.5	24.7	14.3	28.0	4.6	16.0	0	0	33.3	...	41.6	0	0
29	33.3	20.0	10.2	19.4	13.8	24.5	5.0	5.9	...	...	27.2	...	33.3	...	0
30	50.0	20.0	10.5	14.6	18.2	20.0	0	0	...	...	0	...	0	...	0
31	100.0	40.0	16.7	17.2	28.6	20.8	0	0	...	...	0	...	0	...	...
32	100.0	0	25.0	19.0	33.3	21.0	0	0	...	...	0	...	0	...	...
33	...	0	0	26.6	0	28.5	0	0	...	...	0	...	0	...	...

* The earliest age at which a tumor was observed.
† Rats that have never been bred or were found to be sterile.

Several interesting facts are gathered from the computations recorded in Table IV. The average Albany strain rat, once it reaches the age of 18 months, can be expected to live about 4 more months, or 6 months less than the maximal life span for the strain. The breeding females have a greater life expectancy than the nonpregnant rats. The significantly greater life expectation of the breeders from birth can be readily accounted for by the fact that rats are not generally placed in breeding cages before the third month of age, so that the animals included in this group have the advantage of having already survived the hazards of the first 3 months of life. However, the tendency for a longer life expectancy persists through the subsequent periods.

Table III gives the percentages of survivors at the various ages for the colony as a whole, the nonpregnant rats, and for Line 147. It can be seen that if Murray and Hoffman's criterion of the life span is used, namely, "the period of time between birth and that age at which only 1 per cent of the population survives," then the life span of the colony as a whole is 28 months, and that for Line 147 is 26 months.

In considering the longevity of rats it seemed of interest to determine not only the life span, as represented by those rats most able to survive, but rather the mean length of life, which is the average number of months a rat can be expected to live from the beginning of a specific age. The calculation of such a life table is simply explained and clearly illustrated by Dublin (7). Table IV was computed on the basis of life expectation. The number of survivors at the given ages are also shown, to indicate the size of the groups used for the computation.

Finally, the life expectancy of the Line 147 females appears to be consistently lower than for the colony taken as a whole. Thus for both the maximal and average life span the indication is that Line 147 rats, which show the highest tumor incidence of all the lines constituting the colony, tend to be physiologically a little older than the average rat of the same chronological age. The difference, however, is not sufficiently great to compute the tumor incidence on the basis of physiological age (see Murray and Hoffman).

The method of computing a single index of tumor incidence on the basis of chronological age is illustrated in Table V. For example, it was found that 2,787 females had reached the age of 12 months, and that there were 81 females that were first discovered to have a mammary tumor at this age. Inasmuch as 42.3 per cent of a standard population of 10,000 reach the age of 12 months, there would be 4,230 survivors of that age in the standard population. On the basis of the rate per 10,000 calculated from the actual data, it would be expected that 123 of these would develop a mammary tumor ($0.423 \times 291 = 123$). The expected cases can be calculated directly from the proportion $2{,}787{:}81 = 4{,}230{:}x$, where x would equal 123. By adding each of the last two columns and correcting for a population of 10,000 by the proportion given in a footnote to Table V, one may calculate that in a standard population for the ages 5 months and over it can be predicted that 209 new cases of benign mammary tumor will develop among 10,000 nontumorous animals. The rate can be standardized for any age; that of 5 months was chosen because it was the earliest age at which a fibroadenoma has been observed in our colony. Calculations from birth were similarly computed and will be referred to later (Table VII).

By applying this method of calculation to each of the periods analyzed, it is clear from the data in Table VI that the tumor rate for the colony has progressively increased from January, 1940, to October, 1943; that the tumor incidence for Line 147 is higher than that for the whole colony, and that it has increased between August, 1941, and October, 1943. From the ratio of rates it can be seen that in 1940 tumors developed in the colony less than half as frequently as they appear at present in Line 147 (47.2

TABLE III: PERCENTAGE OF SURVIVORS FOR THE VARIOUS GROUPS OF NONTUMOROUS RATS *

	Whole colony	Whole colony, nonbreeders †	Line 147 (breeders and nonbreeders)
Total number of rats	2,665	1,874	453
Age in months			
0	100	100	100.0
1	99.3	99.0	100.0
2	98.5	95.6	97.0
3	92.9	89.8	90.5
4	87.1	82.0	84.4
5	80.5	73.2	79.7
6	70.2	60.3	71.3
7	64.0	53.8	66.4
8	58.4	48.6	61.2
9	54.0	44.7	53.2
10	49.3	40.6	47.9
11	45.8	38.1	45.5
12	42.3	35.4	42.4
13	37.6	31.2	38.0
14	34.9	28.5	35.8
15	31.5	26.3	30.5
16	27.1	22.8	26.3
17	23.6	20.1	21.6
18	20.3	17.4	18.8
19	16.8	14.2	13.5
20	13.4	11.3	10.6
21	10.4	8.4	7.5
22	8.1	6.7	5.3
23	6.1	5.1	3.5
24	4.6	3.8	2.4
25	3.6	3.0	1.5
26	2.4	2.0	0.9
27	1.9	1.7	0.7
28	1.0	0.9	0.2
29	0.8	0.5	0
30	0.6	0.4	0
31	0.2	0.2	0
32	0.1	0.1	0
33	0.08	0.05	0
34	0	0	0

* From the inception of the colony to June, 1942.
† See footnote 2, Table II.

TABLE IV: EXPECTATION OF LIFE IN MONTHS FOR VARIOUS GROUPS OF NONTUMOROUS RATS OF ALBANY STRAIN

	Life expectation from ages					Number of survivors at ages					Age at which 1% of pop. survived, mo.
Months	0	3	6	12	18	0	3	6	12	18	
Whole colony	11.36	9.08	8.47	6.31	3.97	2,665	2,473	1,870	1,125	542	28
Colony nonbreeders *	10.17	8.09	8.22	6.27	3.84	1,874	1,682	1,129	664	326	28
Colony breeders	14.15	11.26	8.83	6.37	4.12	791	791	741	461	216	30
Line 147	11.05	8.48	7.95	5.60	2.96	453	410	323	192	85	26
Line 147 nonbreeders *	9.96	8.15	7.66	5.54	2.59	324	281	206	114	54	26
Line 147 breeders	13.76	10.76	8.36	5.45	3.26	129	129	117	78	31	26

* See second footnote, Table II.

per cent), and that the tumor incidence in the colony as a whole is to date only 65 per cent as great as that of Line 147.

TABLE V: ILLUSTRATIVE TABLE SHOWING AGE DISTRIBUTION OF RATE AT WHICH NEW TUMORS ARE DEVELOPED IN NONTUMOROUS RATS AND METHOD OF STANDARDIZING THE RATES TO OBTAIN A SINGLE INDEX *

Age in months (1)	Survivors to specified ages without tumors (2)	Rats developing new tumors (3)	Rate per 10,000 at which new tumors are developed‖ (4)	Survivors in stand. pop.,† per cent (5)	New rates expected in stand. pop.‖ (6)
5 ‡	4,635	4	9	80.5	7
6	4,279	5	12	70.2	8
7	3,980	1	3	64.0	19
8	3,691	9	24	58.4	14
9	3,464	20	58	54.0	31
10	3,217	48	149	49.3	73
11	2,994	36	120	45.8	55
12	2,787	81	291	42.3	123
13	2,527	65	257	37.6	97
14	2,324	75	323	34.9	113
15	2,105	70	333	31.5	105
16	1,827	94	515	27.1	140
17	1,597	74	463	23.6	109
18	1,356	84	619	20.3	126
19	1,140	66	579	16.8	97
20	940	53	564	13.4	76
21	782	49	627	10.4	65
22	639	44	689	8.1	56
23	492	26	528	6.1	32
24	377	37	981	4.6	45
25	280	31	1,107	3.6	40
26	201	12	597	2.4	14
27	133	9	677	1.9	13
28	93	11	1,183	1.0	12
29	62	5	806	0.8	6
30	48	2	417	0.6	3
31	29	1	345	0.2	1
32	21	0	0	0.1	0
33	15	0	0	0.08	0
34	12	3	2,500	0	0
35	1	1	10,000	0	0
				709.6	1,480

New case rate standardized for ages 5 months and over:
$\frac{70,960}{10,000} = \frac{1,480}{x}$, or 209 per 10,000 nontumorous animals.

* Based on data for whole colony, October, 1943.

† The standard population was obtained from the composite death curve of all the Albany strain nontumorous rats from the inception of the colony to June, 1942.

‡ The earliest age at which a tumor was observed.

‖ The term "new tumors" refers only to the first or original tumors to appear in a given animal, not to any that may appear subsequently, for many rats develop multiple tumors.

The relative progress in increasing the tumor incidence in the colony is apparent to the same degree even when the single index is calculated for a standard population from birth. By using the percentage of survivors in the whole colony (given in Table II) for the ages earlier than 5 months, and the sum of 118,740 substituted for 70,960 in the proportion (footnote, Table V), the standardized tumor rate per 10,000 from birth can be calculated. The comparison of rates computed from birth and from the age of the earliest appearance of a tumor (Table VI) shows that although the absolute values are smaller when the population at the pretumor ages (0 to 5 months) are taken into account, the ratios of the rates remain practically identical with those obtained from the indices for 5 months and over. The figures for the tumor rates from birth are given in case there is an opportunity to compare the tumor incidence in the Albany colony, and in Line 147 specifically, with any other existing group of rats in which fibroadenomas are appearing spontaneously at an age earlier than 5 months.

DISCUSSION

The findings recorded in this paper indicate that the effort to increase the tumor incidence in the Albany strain of rats by close inbreeding has been successful. So far as the authors are aware, the Albany colony is the only fairly large group of rats in which the incidence of spontaneous fibroadenoma has been reported to be of such a high order. Publications from other laboratories have reported an incidence of such tumors in rats of less than 1 per cent of large populations (6, 9). Inasmuch as the Albany colony is the only one for which the incidence has been calculated by methods of computation promising to replace that of simple ratio, it would be interesting to see how the tumor incidence in this colony compares with that in other strains of rats for which comparable methods were used to calculate the tumor incidence.

In contrast to the definite tendency in mice to develop rapidly fatal malignant neoplasms of the mammary gland, the tumor that arises by far the most frequently in the mammary glands of rats is the benign fibroadenoma (or adenofibroma). The morphology of these growths has been described by Heiman (8) and, for the Albany colony in particular, by Wright, Klinck, and Wolfe (14). The tumors grow for the most part rather slowly. Inasmuch as it is the custom in this laboratory to perform a necropsy when they become large and necrotic, it is not possible to state precisely how these tumors affect the life span of the animals bearing them. It was for this reason that the standard population chosen for the computations was calculated from the death rates of the nontumorous animals exculsively; and, indeed, it probably represents more closely a sample of an average rat colony than otherwise would have been the case.

The fact that these benign tumors have appeared more frequently in rats that have not been pregnant seems to be of unusual significance. The findings

appear to indicate that the absence of the higher hormone levels that presumably characterize the pregnant state, and stimulate a well-developed lobule-alveolar system in the mammary glands, actually constitutes a condition favorable for the development of the tumors. This observation immediately suggests that one reason for the high incidence of tumors in the colony as a whole is the endocrine dysfunction, which results in a failure of ovulation and a consequent decrease in pregnancies. Generally this ovarian deficiency, which we have already suggested is due secondarily to an anterior lobe dysfunction, tends to be more severe in rats developing tumors. Thus in this colony there seems to be a good correlation between the development of benign mammary tumors and hypofunction of the reproductive system.

TABLE VI: CORRECTED TUMOR RATES PER 10,000 ON THE BASIS OF A STANDARD POPULATION, AS ILLUSTRATED BY TABLE V

		Whole colony								Line 147					
		Jan., 1940		Aug., 1941		June, 1942		Oct., 1943		Aug., 1941		June, 1942		Oct., 1943	
Age in months	Survivors in stand. pop., per cent	Tumor rate per 10,000	Expected tumors in stand. pop.	Tumor rate per 10,000	Expected tumors in stand. pop.	Tumor rate per 10,000	Expected tumors in stand. pop.	Tumor rate per 10,000	Expected tumors in stand. pop.	Tumor rate per 10,000	Expected tumors in stand. pop.	Tumor rate per 10,000	Expected tumors in stand. pop.	Tumor rate per 10,000	Expected tumors in stand. pop.
5 *	80.5	22	18	15	12	10	8	9	7	0	0	0	0	0	0
6	70.2	6	4	10	7	11	8	12	8	16	11	10	7	7	5
7	64.0	7	5	4	2	3	2	3	19	0	0	0	0	0	0
8	58.4	25	15	24	14	22	13	24	14	41	24	35	20	40	23
9	54.0	65	35	53	29	41	22	58	31	47	26	37	20	75	41
10	49.3	147	73	118	58	134	66	149	73	278	137	253	125	272	134
11	45.8	81	37	113	52	108	50	120	55	485	222	261	120	241	110
12	42.3	244	103	213	90	299	126	291	123	440	186	644	272	521	212
13	37.6	181	68	196	74	276	104	257	97	730	275	674	253	502	189
14	34.9	218	76	211	74	268	94	323	113	303	196	438	153	537	187
15	31.5	226	71	322	101	358	113	333	105	159	50	428	135	405	128
16	27.1	254	68	354	96	352	95	515	140	727	197	397	108	840	228
17	23.6	311	73	357	84	421	98	463	109	196	46	734	173	787	186
18	20.3	510	104	523	106	596	121	619	126	682	139	906	184	889	180
19	16.8	519	87	584	98	674	113	579	97	884	149	800	134	697	116
20	13.4	375	28	402	54	518	69	564	76	667	89	893	120	634	85
21	10.4	574	60	418	44	526	55	627	65	417	43	807	84	1,107	115
22	8.1	0	0	642	52	605	49	689	56	0	0	494	40	684	55
23	6.1	469	29	476	29	436	27	528	32	1,538	95	612	37	1,172	69
24	4.6	435	20	747	34	626	29	981	45	2,000	92	2,070	95	2,130	72
25	3.6	714	26	619	22	706	25	1,107	40	0	0	1,111	40	1,739	63
26	2.4	1,667	40	580	14	377	9	597	14	0	0	0	0	1,250	30
27	1.9	1,667	32	790	15	357	7	677	13	0	0	0	0	1,071	20
28	1.0	0	0	870	9	176	2	1,183	12	0	0	0	0	1,333	13
29	0.8	0	0	668	5	204	2	806	6	...	...	...	...	2,727	22
30	0.6	0	0	0	0	526	3	417	3	...	...	...	...	0	0
31	0.2	0	0	0	0	834	2	345	1	...	...	...	...	0	0
32	0.1	10,000	10	4,000	4	2,500	3	0	0	...	...	...	...	0	0
33	0.08	0	0	0	0	0	0	0	0	...	...	...	...	0	0
	709.6		1,082		1,179		1,315		1,480		1,887		2,120		2,283
Corrected tumor rate for ages 5 mos. and over		152		166		185		209		266		299		322	
Ratio of rates			47.2		51.5		57.4		65.0		82.6		94.0		100

* The earliest age at which a tumor was observed.

TABLE VII: COMPARISON OF STANDARDIZED TUMOR RATES PER 10,000 FROM BIRTH AND FROM AGE OF EARLIEST APPEARANCE OF TUMOR

	Total per cent survivors in stand. pop.		Whole colony				Line 147		
			1940	1941	1942	1943	1941	1942	1943
Birth	1,187.4	Tumor rate	91	99	111	125	159	179	192
		Ratio	47.4	51.6	57.8	65.2	82.9	93.3	100
5 mos.	709.6	Tumor rate	152	166	185	209	266	299	322
		Ratio	47.3	51.6	57.5	65.0	82.6	92.9	100

SUMMARY AND CONCLUSIONS

The incidence of spontaneous benign mammary tumors (fibroadenomas) in the Albany colony of rats and in one subline (Line 147) yielding the greatest number of tumors, was calculated by two methods: (a) the percentage of tumor bearers among animals that live to the beginning of each age group and (b) corrected tumor rates per 10,000, using a standard population.

The tumor rates were computed for 4 successive years in order to determine whether the frequency of tumor development was increasing or decreasing. It was found that the tumor incidence, both for the colony as a whole and for Line 147 specifically, had increased from January, 1940, to October, 1943. From the ratio of tumor rates obtained for the various periods it was shown that in 1940 tumors developed in the colony as a whole less than half as frequently as in 1943 for Line 147, and in 1943 the incidence for the Albany colony was a little more than three-fifths that of Line 147. Thus the effort to increase the tumor incidence in this colony by close inbreeding seems to have been successful.

It was further found that the tumor incidence, calculated by the first method given above, was in general higher for Albany females that had never been pregnant than for the breeding animals.

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The Non-Heme Iron Content of the Tissues of Mice of High-Cancer and Low-Cancer Strains*

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Cancer is essentially a disease of later life. This holds good for most types of tumors in all species that have been investigated. It is apparent that studies of the factors involved in the aging of animals may be of great interest when considered in connection with the spontaneous appearance of cancer. Many investigations of aging organisms have been made, yet, as a conclusion to his review of the chemical aspects of aging, McCay (4) admits—"almost nothing is known of the biochemistry of ageing."

In view of the almost complete absence of knowledge of the biochemical factors involved in the process of aging the experiments of Zondek and Karp (5) on the relationship between iron and the aging of cells are particularly interesting. They found that the non-heme iron content of the exsanguinated epithelial tissues (liver, kidneys, and testis) of 5 species of animals was relatively constant, but that at a definite period of life and within a short time the iron content of the epithelial organs increased up to 200 per cent. The same iron content is then maintained until death. The whole increase in iron content takes place during the middle period of life. The longer the life span of the animal the later does the change occur. In the rat, for example, the iron content of the kidney and testis does not alter throughout the first 10 months of life. The increase begins after about 15 months and reaches a maximum at 18 months. For the remainder of the rat's life (3 to 4 years in all) the iron content remains the same. Bruckmann and Zondek (1) investigated the non-heme iron content of human organs. The materials consisted of postmortem specimens of liver and kidney. A characteristic life curve was found for the content of these organs. The course of this life curve was so similar to the familiar life curve for hemoglobin that these workers concluded there must be a connection. They were also of the opinion that the newly acquired iron present in aging cells is probably a compound other than those already present.

These observations seemed to offer a relatively simple method of estimating the "physiological age" of mice whose susceptibility to spontaneous cancer of a certain type (mammary carcinoma) was known. It seemed possible that strains differing in susceptibility to spontaneous cancer might show different physiological ages, as measured by the deposition of iron in the organs, at the same chronological age. The data in the present paper are the result of such an investigation. As a secondary objective the simultaneous construction of life curves for tissue non-heme iron and blood hemoglobin (3) would clearly be of value in substantiating Zondek's assertion that the increase of non-heme iron in aging cells is correlated with their aging and is not merely the result of concomitant blood pigment destruction. In the experiments of Zondek and Karp only a small number of animals was used, 125 of all species. The greatest number of measurements were made on rats but no indication of sex is given in the protocols, although internal evidence suggests that the animals were exclusively males.

MATERIALS AND METHODS

The mice used in these experiments were of 3 strains and both sexes of each strain were investigated. Mice of the RIII and C3H strains (high-mammary-cancer) and CBA (agouti) (low-mammary-cancer) mice were employed. The kidney was chosen as being a well-defined organ giving easy and reproducible dissection for weighing and estimation of the non-heme iron. The mice were killed at selected ages and the kidneys immediately removed and weighed. Non-heme iron was then extracted by the hot pyrophosphate method of Bruckmann and Zondek (2). The iron in the extracts was determined by measuring the color developed with thioglycolic acid by means of a Spekker photoelectric absorptiometer.

RESULTS

The results obtained on male and female mice of the 3 strains are shown in Tables I to VI. The combined values are illustrated graphically in Fig. 1. All iron values are expressed in micrograms per gram of fresh kidney tissue.

* Because of the difficulties of international communication the authors have not read proof of this article.

TABLE I: NON-HEME IRON IN THE KIDNEYS OF MALE CBA MICE (AGE GROUPS)

Age, days	μgm. Fe/gm.	No. of mice
0-100	50.0	35
100-200	43.0	26
200-300	64.0	11
300-400	59.0	13
400-500	57.0	9
500-600	44.0	12
600-700	55.0	18
700-800	60.0	7

TABLE II: NON-HEME IRON IN THE KIDNEYS OF FEMALE CBA MICE (AGE GROUPS)

Age, days	μgm. Fe/gm.	No. of mice
0-100	52.0	13
100-200	52.5	9
200-300	69.0	5
300-400	97.0	8
400-500	86.5	9
500-600	89.0	13
600-700	96.0	20
700-800	98.0	2
800-900	98.5	2

TABLE III: NON-HEME IRON IN THE KIDNEYS OF MALE C3H MICE (AGE GROUPS)

Age, days	μgm. Fe/gm.	No. of mice
0-100	35.0	5
100-200	42.5	19
200-300	52.0	16
300-400	52.0	15
400-500	60.0	7
500-600	81.0	2

TABLE IV: NON-HEME IRON IN THE KIDNEYS OF FEMALE C3H MICE (AGE GROUPS)

Age, days	μgm. Fe/gm.	No. of mice
100-200	45.0	3
200-300	42.0	5
300-400	50.0	17

TABLE V: NON-HEME IRON IN THE KIDNEYS OF MALE RIII MICE (AGE GROUPS)

Age, days	μgm. Fe/gm.	No. of mice
0-100	42.0	4
100-200	49.5	9
200-300	41.0	5
400-500	59.0	19
550-650	47.0	5

TABLE VI: NON-HEME IRON IN THE KIDNEYS OF FEMALE RIII MICE (AGE GROUPS)

Age, days	μgm. Fe/gm.	No. of mice
300-400	74.0	9
400-500	55.5	19
500-650	55.5	8

DISCUSSION

Male mice of the CBA strain show little variation throughout life. The extreme limits for any group of mice are 43-64 μgm. The females of this line, however, show a very definite increase in tissue non-heme iron between 300 and 400 days of life. At this period the kidney non-heme iron rises suddenly to a value approximately twice its initial value, and is maintained at this high level for the rest of the animal's life.

In males of the RIII strain there is some increase in non-heme iron in later life. Values for young RIII females are lacking but the values in older mice are not very high. Clearly, it will be desirable to study younger females of this strain, when they become available, in order to test more adequately the reality of the apparent fall in non-heme iron from the relatively high value shown between 300 and 400 days to the lower value maintained up to 650 days of life.

In the C3H strain mice there is no great difference in tissue non-heme iron between young and old mice of either sex. Both sexes show some increase with advancing age.

TABLE VII: KIDNEY NON-HEME IRON: VALUES FOR BOTH SEXES OF 3 STRAINS IN YOUNG AND OLD MICE

Strain	Sex	Under 300 days, μgm. gm.	Over 300 days, μgm. gm.
CBA	Male	50	55
CBA	Female	55	93
RIII	Male	45	57
RIII	Female	..	60
C3H	Male	45	57
C3H	Female	43	50

These relationships are made clear in Table VII, in which a division of all the animals used is made at 300 days of age, that is, the age at which the females of the CBA strain begin to show a striking increase in the non-heme iron content of the kidneys.

It will be seen that in mice under 300 days of age there is little variation with respect to strain or sex, and that the same holds good for animals over 300 days with the exception of female CBA mice. Excluding CBA females over 300 days, the mean non-heme iron contents of the kidneys are, for all animals under 300 days, 47.6 μgm., and for all animals over 300 days, 55.8 μgm.

If the average water content of kidney is taken as 80 per cent these values are equivalent to 238 mgm. per kilo of dried tissue and 279 mgm. respectively. These figures may be compared with Zondek and Karp's values for young rats (140 mgm./kilo) and for old rats (335 mgm./kilo).

In each of the six groups of mice studied (both sexes of 3 strains) the tissue non-heme iron shows an increase with increasing age. In no group, however,

with the exception of CBA female mice, is this increase so distinct or so rapid as might have been expected from Zondek's results on other species of animals.

A test of the methods described in this paper on rats left no doubt as to the reality of the effect described by Zondek and his collaborators. In 12 young male rats (4 to 6 weeks) the average kidney non-heme iron was 31 μgm./gm. of fresh tissue and in 13 young female rats of the same age the value was again 31 μgm./gm. In old rats (2 years) the average iron

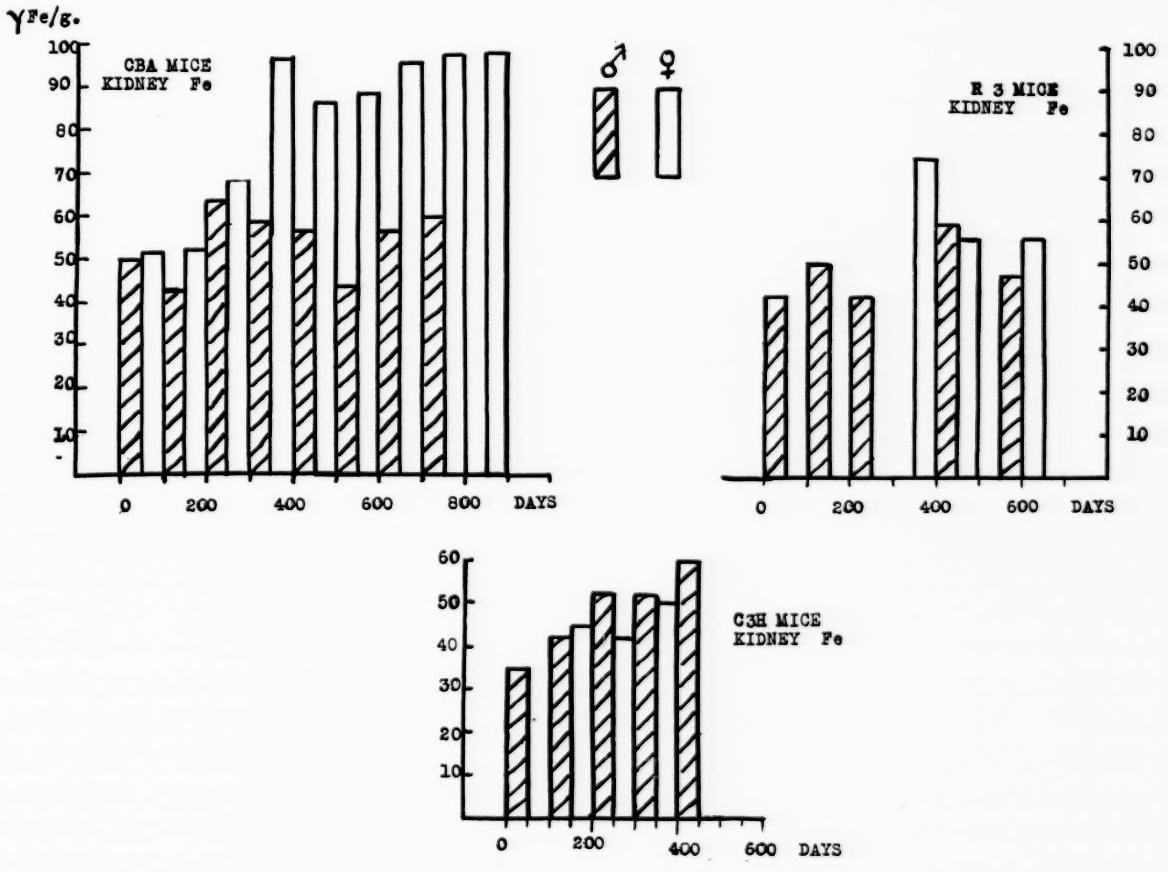

FIG. 1.—The non-heme iron content of the kidneys of three strains of mice at different ages.

contents for 15 males and for 11 females were 76 μgm./gm. and 103 μgm./gm. respectively. The values given by Zondek and Karp for young and for old rats are equivalent to 28 μgm./gm. and 77 μgm./gm. respectively when calculated to a fresh tissue basis. These are in remarkably good agreement with those cited above for our male rats. The considerable difference between the iron contents of the kidneys of old male rats (76 μgm./gm.) and old female rats (103 μgm./gm.) in our experiments is highly significant ($P<0.01$).

The present results support Zondek's view that the additional iron acquired by aging cells is not derived from blood pigment destruction. As shown in an earlier paper (3) the blood-hemoglobin life curve for CBA female mice falls very slowly over a considerable range of the animal's life span. Unless a sudden change occurs in the blood hemoglobin turnover rate at about 300 days of age it is difficult to attribute the sudden rise in tissue iron that begins at this time to the liberation of iron from the blood hemoglobin. Furthermore, in RIII female mice the sudden onset of a blood pigment destructive process (3) that rapidly lowers the hemoglobin content of the blood at about 400 days of age is not followed by the appearance of any notable increase in the non-heme iron of the tissues.

The conclusion to be drawn from the present experiments is that, if one accepts Zondek's criterion that physiological aging of cells is indicated by their acquisition of non-heme iron, there is no indication that the cells of mice of high-cancer strains are physiologically older at the same chronological age than the corresponding cells of mice of a low-cancer strain.

SUMMARY

In some species the aging of an animal is accompanied by a definite increase in the non-heme iron content of the tissues. Estimations of non-heme iron in the kidneys of mice of 2 high-mammary-cancer strains (RIII and C3H) and 1 low-mammary-cancer

strain (CBA) were made with a view to detecting differences in physiological age between high- and low-mammary-cancer mice at the same chronological ages. No such differences were found. Female mice of the CBA strain showed a considerable increase in kidney non-heme iron between 300 and 400 days of age. With this exception no great difference was found between young and old mice of either sex in any of the mice investigated.

The great increase in the non-heme iron content of the tissues of old rats has been confirmed for both sexes. The non-heme iron content of the kidneys of old female rats is higher than that of the kidneys of old male rats to a highly significant extent.

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The Hemoglobin Content of the Blood of Mice of the RIII and CBA Strains*

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The hemoglobin content of mouse blood has been little investigated, and until the recent outstanding work of Strong and his collaborators (2, 3, 4, 5, 6) practically nothing was known about the normal physiological variation of mouse blood pigment with such factors as age and strain. Strong's interest in the hemoglobin content of the blood of mice susceptible to spontaneous cancer was aroused by the fact that agents affecting the cell in its oxidation-reduction relationships are capable of eliciting profound effects on development. In a study of the hemoglobin life curves in different strains of mice Strong made the important observation that there is a precocious drop in the hemoglobin level in those mice that are known to be more susceptible to the development of spontaneous cancer as compared with those that are more resistant.

Parsons (1) has shown that the induction of cancer by some carcinogens is preceded by the deposition of iron in certain tissues. A similar effect obtains in mice bearing spontaneous tumors (8). Carcinogenic hydrocarbons that have been rendered water-soluble by conversion into *endo*-succinates have a strong hemolytic action *in vitro* (9).

In connection with further investigation of the iron content of mouse tissues (10) it was considered necessary to obtain concurrently data on the hemoglobin content of the blood. Such data are recorded in the present paper. Since Strong's experiments were confined to female mice the present work was extended to include estimations on male mice from a high- and from a low-cancer strain.

MATERIALS AND METHODS

The mice used in these experiments were of two strains. CBA (agouti) mice were selected as the low-mammary-cancer strain in order to afford a basis of comparison with Strong's results. As a high-mammary-cancer strain mice of the RIII line were chosen. This strain was developed at the Radium Institute of Paris by Mme. Dobrovolskaia-Zavadskaia. The incidence of spontaneous mammary cancer in the females of this line has been given as about 72 per cent of all females living to about 7 months. In the mice of the colony used in these experiments the spontaneous incidence was high. Up to 1940 the incidence approached 100 per cent in female mice living at 6 to 8 months of age. Since that time there has been a gradual lengthening of the latent period, at the moment unexplained, which is now over one year (400 to 450 days).

All mice used for hemoglobin estimations were kept on the same adequate diet. No mouse was used for more than two bleedings, and a period of at least 12 weeks was allowed to elapse between two such measurements. The animals were removed from food 12 hours before bleeding.

The oxyhemoglobin content of blood can be rapidly and accurately estimated with the help of a photoelectric colorimeter. Szigeti (7) has described a suitable method for use with the Hilger photoelectric absorptiometer and this method was employed. Calibration of the instrument was carried out with a blood sample in which the pigment content had been determined spectrophotometrically. Blood samples were drawn from the tail after amputation of about 1 to 2 mm. of the tip. The sample was easily collected by inserting into the blood droplet the tip of a standard 0.1 ml. blood pipet previously rinsed with saturated sodium oxalate solution. Duplicate samples of 0.05 ml. were taken on each occasion of bleeding. The contents of the pipet were blown out into 10 ml. of 0.04 per cent ammonia solution. The ammoniacal solution was well shaken and transferred to the cells of the absorptiometer. The average time between dilution and reading in the colorimeter was 15 minutes. The method was tested for reproducibility by measuring the hemoglobin content of the blood of the same animal on successive samples taken at intervals of about 1 minute. Agreement between duplicates was good.

RESULTS

Estimations were carried out on male and female mice of both strains at different ages. The values ob-

* Because of the difficulties of international communication the authors have not read proof of this article.

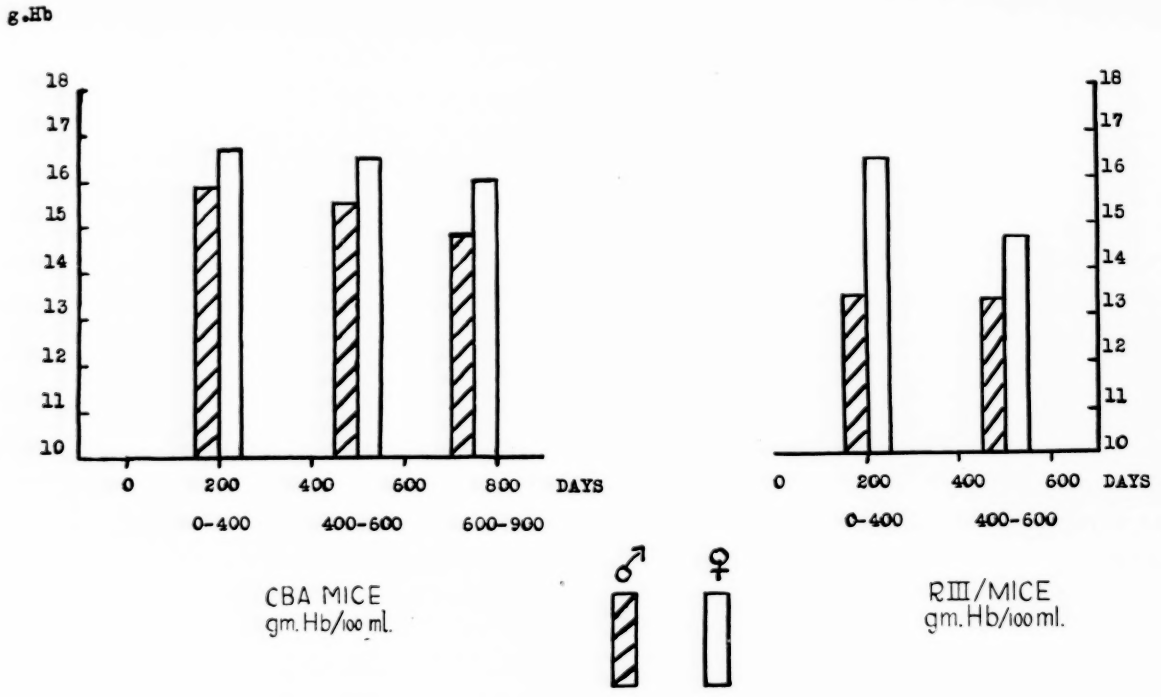

FIG. 1.—Blood hemoglobin at different age levels in mice of the CBA and RIII strains.

tained are set out in Tables I to IV. Table V and Fig. 1 show, in a consolidated form, the results obtained on all the animals used.

TABLE I: HEMOGLOBIN CONTENT OF THE BLOOD OF MALE CBA MICE AT DIFFERENT AGES

Age group, days	gm. Hb/100 ml.	No. of mice	Range
250-300	16.0	4	15.6-16.4
300-350	16.0	4	15.4-17.0
350-400	15.7	10	14.5-16.8
400-450	16.0	12	14.3-17.5
450-500	15.3	15	10.4-17.7
500-550	15.5	9	13.7-17.1
550-600	15.1	5	14.4-15.8
600-650	14.8	24	7.7-18.8
650-700	14.8	9	10.5-18.7
700-750	15.0	4	13.9-17.3
750-800	15.0	4	12.5-17.1
800-900	15.0	2	14.1-15.8

TABLE II: HEMOGLOBIN CONTENT OF THE BLOOD OF FEMALE CBA MICE AT DIFFERENT AGES

Age group, days	gm. Hb/100 ml.	No. of mice	Range
150-200	16.5	4	15.6-17.2
200-250	16.6	9	15.5-18.3
250-300	17.7	3	16.8-19.3
300-350	17.3	7	16.3-18.3
350-400	16.5	32	12.8-18.7
400-450	16.9	19	15.4-18.3
450-500	16.5	16	13.8-18.1
500-550	16.4	7	13.8-17.7
550-600	15.7	8	14.3-16.8
600-650	16.0	19	14.1-17.2
650-700	16.0	5	14.6-17.9
700-900	15.7	7	14.0-17.5

TABLE III: HEMOGLOBIN CONTENT OF THE BLOOD OF MALE RIII MICE AT DIFFERENT AGES

Age group, days	gm. Hb/100 ml.	No. of mice	Range
100-150	15.1	3	14.7-15.4
200-250	14.0	12	12.3-15.3
250-300	13.5	13	7.2-15.1
300-350	13.5	20	7.6-15.7
350-400	12.3	11	8.3-15.2
400-450	13.5	17	10.1-15.6
450-500	13.8	9	11.9-14.9
500-550	12.4	6	7.6-14.6

TABLE IV: HEMOGLOBIN CONTENT OF THE BLOOD OF FEMALE RIII MICE AT DIFFERENT AGES

Age group, days	gm. Hb/100 ml.	No. of mice	Range
0-100	16.5	3	16.2-16.8
200-250	16.7	9	15.6-17.5
250-300	15.8	1	
300-350	16.1	23	13.8-17.5
350-400	16.8	14	12.7-23.9
400-450	14.1	15	9.7-16.7
450-500	15.3	21	13.0-17.7
500-600	14.7	6	12.7-18.7
Over 600	14.4	2	

DISCUSSION

Male mice of the CBA strain have hemoglobin contents of whole blood of 15.9 gm. at 0 to 400 days, 15.5 gm. at 400 to 600 days, and 14.8 gm. at 600 to 900 days. Thus, from about the 200th to the 700th day of life there is a fall in hemoglobin of 1.1 gm. per 100 ml. Females of this strain show values of

16.7 gm., 16.5 gm., and 16.0 gm. at the same respective average ages—a decrease of 0.7 gm. per 100 ml. over about 500 days. At all ages the blood hemoglobin is higher in females than in males. The sexual difference is about 5 per cent in the lower age group and increases to 8 per cent at 600 to 900 days of life. It may be noted that the value of 16.7 gm. per 100 ml., determined in these experiments for female breeding CBA mice at 0 to 400 days, is comparable with the value found by Strong for CBA female breeders at 200 days of age, *i.e.,* 16.3 gm.

In male mice of the RIII strain there is no significant difference between mice of the age groups 0 to 400 days and 400 to 600 days. The hemoglobin contents are 13.45 gm. and 13.35 gm. respectively. Females of this line, however, show a notable difference. The hemoglobin value of 16.4 gm. at 0 to 400 days falls to 14.7 gm. at 400 to 600 days, a decrease of 1.7 gm. This fall occurs quite sharply at or about the 400th day since the average value for 25 mice between 324 and 395 days is 16.4 gm., which is the same as the average for all mice under 400 days, while the average value for 21 mice of ages between 400 and 457 days is 14.4 gm., which is practically the same as the average for all RIII mice over 400 days old. Thus the rapid fall in hemoglobin occurs in the period 360 to 430 days. The sexual difference in hemoglobin is very definite in the RIII strain. At all ages the values for the females are higher than those for males. The sexual difference decreases with increasing age of the animals, being 22 per cent at 0 to 400 days and 10 per cent at 400 to 600 days.

These results are in complete agreement with the findings of Strong on his strain A (high-mammary-cancer) mice. The fall in hemoglobin in female RIII mice is not so great numerically as that found by Strong in strain A mice, but appears to take place even more precipitously. Whereas females of strain A showed a fall of 28 per cent of the initial hemoglobin content in 400 days, females of the RIII strain show a decrease of about 10 per cent in 70 days. Furthermore, the sharp decrease occurs at approximately the age (400 days) at which mammary cancer begins to appear in the colony of RIII female mice used in these experiments. It should be emphasized that no mouse bearing a palpable tumor was used for hemoglobin determinations.

Table V: Hemoglobin Content of the Blood of Male and Female Mice of the CBA and RIII Strains at Different Ages

Age group, days	Strain	Sex	gm. Hb/100 ml.	No. of mice	Standard deviation	Standard error
0-400	CBA	M	15.9	18	0.76	0.17
0-400	CBA	F	16.7	55	1.31	0.18
0-400	RIII	M	13.45	59	2.03	0.26
0-400	RIII	F	16.4	50	1.62	0.23
400-600	CBA	M	15.5	41	1.52	0.24
400-600	CBA	F	16.5	50	1.14	0.16
400-600	RIII	M	13.35	32	1.74	0.31
400-600	RIII	F	14.7	42	1.97	0.30
600-900	CBA	M	14.8	43	2.48	0.38
600-900	CBA	F	16.0	31	1.03	0.19

SUMMARY

Blood hemoglobin has been estimated in both sexes of two strains of mice. In both strains (CBA, low-mammary-cancer; RIII, high-mammary-cancer) the concentration of blood pigment is higher in female mice than in male mice at all ages. Female mice of the RIII strain show a rapid fall of blood hemoglobin between the ages of 360 and 430 days. This fall amounts to about 10 per cent of the blood pigment initially present and occurs at the age at which spontaneous mammary carcinoma normally begins to appear in female mice of this strain.

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Factors Affecting Carcinogenesis

II. Incorporation of 3,4-Benzpyrene in Media Containing Purified Lecithin or Cephalin*

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In a previous communication (3) we described the inhibition of carcinogenic activity of 3,4-benzpyrene when a mixture of lipids prepared from ox brain was employed as solvent. Out of an effective total of 29 mice receiving subcutaneous implants of pellets of these lipids containing 0.3 mgm. benzpyrene only 1 mouse developed a tumor, whereas among an effective total of 40 control mice injected with the same dose dissolved in vegetable oils 30 tumors were obtained. The method of preparation suggested that the main constituents of the lipid mixture were phospholipids and cholesterol. It was decided to repeat the experiment with purified preparations of lecithin and cephalin and to study at the same time the rate of elimination of benzpyrene from the animal.

The fact that the inhibitory effects observed were shown by fats and lipids from animal sources only, and were absent from vegetable oils, suggested that they might be connected with the presence of one of the unsaturated fatty acids such as arachidonic or clupanodonic acid, which so far have been found only in animal fats. As cod liver oil is a particularly rich source of these unsaturated fatty acids, we tested the hypothesis by using cod liver oil as the solvent for a series of benzpyrene injections. Hartwell's catalogue (6) contains only one instance where cod liver oil has been employed as vehicle, a reference to a remark of Burrows (2), who found no tumors in 10 mice injected subcutaneously with 1,2,5,6-dibenzanthracene (amount not stated). Similar experiments with fresh pork fat, olive oil, or sperm oil as vehicles also gave negative results.

A fourth group of mice, which served as a control experiment, was injected with benzpyrene dissolved in tricaprylin.

* Because of the difficulties of international communication the authors have not read proof of this article.

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EXPERIMENTAL

Preparation of cephalin and lecithin.—Cephalin was prepared from ox brain according to the method of Folch and Schneider (5). After four precipitations from ether or light petroleum by alcohol, followed by emulsification with hydrochloric acid, precipitation by acetone, and finally solution in light petroleum and ether, with precipitation by acetone from each of these solvents, cephalin was obtained as a buff-colored powder that gave the following analyses: total N, 1.87 per cent; amino N by nitrous acid, 1.59 per cent; P, 3.88 per cent; ratio amino N/total N, 0.85; ratio N/P, 1.06. It may be mentioned that, according to Folch (4), cephalin of approximately this degree of purity consists of several fractions, including phosphatidyl inositol, phosphatidyl serine, and phosphatidyl aminoethanol.

Lecithin was obtained from the alcoholic mother liquors from which the cephalin had been isolated as described. These were concentrated *in vacuo* and the lecithin was purified by four successive precipitations as the cadmium chloride compound (9) followed by decomposition by ammoniacal methanol, emulsification in dilute acetic acid, and precipitation by acetone. The product was a pale yellow, waxy solid. Analysis: total N, 2.05 per cent; amino N, 0.09 per cent (this value included residual traces of NH_3); P, 3.70 per cent.

Medicinal cod liver oil.—A commercial product of Messrs. Wilkinson and Simpson, Newcastle upon Tyne.

Tricaprylin.—The sample, which had a melting point of 8° to 9° C., was prepared by the method of Hershberg (7).

Subcutaneous administration.—Lecithin and cephalin were mixed with an equal weight of tricaprylin containing 0.2 per cent benzpyrene, but even so a thick paste resulted that was not suitable for injection. The technic of implanting pellets was therefore resorted to, as previously described, the only

modifications being the following: The skin of the midback area was epilated with 40 per cent barium sulphide paste a few days before the experiment. The incision was closed with Michel clamps, which were removed after 25 days.

The mixture of cephalin and tricaprylin liquified on heating to about 60° C. and could be squeezed through a warm syringe. Before this was found, about half of the animals in this group had already received pellets. The remainder were injected.

Cod liver oil and tricaprylin, both containing 0.1 per cent benzpyrene, were delivered into the subcutaneous tissue of the interscapular region through a thin two-inch needle, which was inserted full length from a point above the tail.

As before, the estimated weight of a pellet was 0.3 gm. and the volume of the liquid injections was 0.3 ml. The dose of benzpyrene was therefore 0.3 mgm. in each case.

Owing to difficulties in the supply of mice, mainly females of mixed stock that had previously been used for breeding were available and the scope of the experiment was further limited by the amount of pure lipid material that had been prepared. Twenty animals of each group were kept for the observation of tumor development, while 10 to 13 of each group were destined for analyses of benzpyrene at weekly intervals. A number of mice of the lecithin and cephalin groups that died within 24 hours after operation were replaced on the following day. The bodies were analysed for benzpyrene.

Estimations of benzpyrene were made by the method of Weil-Malherbe (12).

Observations during latent period.—As far as could be judged by inspection and by examination in filtered ultraviolet light there was no serious primary leakage in any of the groups. With both phospholipids, however, and especially in the case of cephalin, the fur assumed a greasy appearance everywhere, not only in the neighborhood of the site of application. Lumps were not observed in the phospholipid groups, but there was a fairly late appearance of sloughs and ulcers in some animals of the lecithin group. These were observed after 3 weeks in 10 of 22 animals. In the group of animals that received cephalin by injection small sloughs were observed in 4 of 10 animals in the second week; 4 sloughs and 1 ulcer were observed in the group of 10 animals with implanted cephalin pellets, also in the second week. In the group of animals injected with tricaprylin neither lumps nor ulcers were observed, whereas there was a number of lumps in the cod liver oil group. These lumps appeared after about 2 to 3 months and remained stationary, in some cases to resume growth later and develop into a tumor, in others to regress after many months, and in some cases to persist until death. On postmortem inspection they were found to consist of encapsulated, yellow, putty-like material. In 2 cases rapidly growing tumors developed without previous appearance of stationary lumps. No ulceration occurred in this group.

RESULTS

Tumor incidence.—The results are summarized in Table I. The incidence of local tumors in the control experiment was lower than had been anticipated from the results of the preceding study (3), where the same dose caused the appearance of 90 per cent tumors in the group injected with sesame oil as solvent. It is unlikely that the difference is due to any solvent effect, but it may be connected with the fact that in the present series mainly female mice were used, which are known to be less susceptible to subcutaneous tumor induction than male mice (8). But this does not adversely affect the present experiment, whose groups are comparable with each other. It is obvious from an inspection of the table that there are two main groups, one with low tumor incidence consisting of the two phospholipid groups, and one with high tumor incidence comprising the tricaprylin and the cod liver oil group. The results within each of these two main groups are practically identical. However, there is a difference between the tricaprylin and cod liver oil groups concerning the latent period; tumors in the second group appeared about 2 months earlier than in the first group.

In the tricaprylin group 2 tumors were observed remote from the site of injection and one such tumor appeared in the cephalin group. In a female mouse of the latter group a tumor arose that had the localization of a spontaneous mammary carcinoma. The microscopic examination, however, suggested that it might be a sarcoma rather than a carcinoma, and it was therefore grouped as a local tumor.

Elimination of benzpyrene.—It may be assumed that the process of elimination in each mouse follows the course of a monomolecular reaction, *i.e.,* that the rate of disappearance is proportional to the concentration. Berenblum and Schoental (1) have indeed obtained evidence that this is the case. Hence the constant

$$K = \frac{1}{t} \log \frac{a}{S}$$

was calculated, where t = the time in days, a = the initial quantity of benzpyrene (300μgm.), and S = μgm. benzpyrene left after t days. Thus a value is obtained that enables a quantitative comparison to be made between different animals at different times. A value of 0.3 μgm. benzpyrene was taken as the lower limit of sensitivity. Although the error may be great at this level, a result of 0.3 μgm. benzpyrene provides at

least qualitative evidence for the presence of benzpyrene. Results below this level are marked < 0.3 μgm. in Table II, and the K-value for $S = 0.3$ μgm. and the respective value of t is entered with the prefix $>$. In the lecithin group the last measurable observation was made on the 47th day. For all the following analyses a value of K was assumed greater than that which would correspond to a value of 0.3 μgm. on the day of the first negative analysis. A similar procedure was followed for the cephalin group; a value greater than that corresponding to $S = 0.3$ μgm. on the day of the first negative analysis following the last measurable observation was assumed for all following analyses. However this procedure is not exact enough to permit of any statistical computation; the calculation of means was therefore omitted in these two groups, and the rate of elimination is to be judged from the time that marks the last measurable observation of benzpyrene.

The results (Table II) are to be regarded as of provisional and exploratory character only. There is a considerable individual variation of K-values, even within the groups. Partly, at least, these variations are due to leakage, which would not have been suspected without analyses. In Table III the results of recovery experiments, 18 hours after subcutaneous application, are shown. Nine mice of the lecithin group and 2 of the cephalin group were found dead on the morning after the operation and are included in the table, although the exact time of their deaths is uncertain; some presumably died from an overdose of anesthetic without regaining consciousness. In these cases leakage would be expected to be smaller than in animals that moved about before the wound had had time to close. Although great care was taken during the injection of cod liver oil and tricaprylin, this did not preclude the occurrence of some leakage even in these groups; the divergence of results within both these groups can hardly be interpreted differently.

Other factors besides leakage may account to some extent for the variations of K, such as differences of the exact site of injection; smaller or greater dispersal of the injected solution; and, finally, truly individual factors such as different tissue responses (various degrees of encapsulation, vascularization, phagocytic infiltration, etc.).

As the risk of leakage is probably greater in the implantation experiments than in the injection experiments all the analyses of the cephalin group were done on injected animals.

In spite of the large individual variations the means of both the cod liver oil group and the tricaprylin group are significant. The first value of the cod liver oil series shows an especially large divergence from the other values. It must be suspected that this is due to leakage. This result has therefore been omitted from the calculation of the mean and standard deviation.

TABLE I: TUMOR INCIDENCE

Solvent	Number of mice alive at appearance of 1st tumor (4 months)	Tumors: Number, site, histology	Tumors: Sex of mouse	Tumors: Latent period, months [4]	Time of death of nontumor mice, months [4]	Number of mice alive after 14 months	Percentage incidence of local tumors
Lecithin	22 (20 ♀, 2 ♂)	2 loc.[1] sarcomas	2 ♀	6.0, 8.2	4.2, 7, 7, 8, 8, 8.2, 9.3, 9.5, 10.2, 12, 12.5, 13	8	9
Cephalin	19 (17 ♀, 2 ♂ 9 P.[2], 10 I.[3])	1 loc. sarc. 1 mammary tumor [5] 1 lymphoblastoma of liver	♀ I.[3] ♀ I.[3] ♂ P.[2]	6.6 4.6 8.6	4.3, 4.3, 7, 8.6, 10, 11, 11	9	9.5
Tricaprylin	18 (14 ♀, 4 ♂)	6 loc. sarc. 1 pulm. adenocarcinoma 1 mediast. lymphoblastoma	4 ♀ 2 ♂ ♀ ♀	5.5, 6.0, 6.5 6.5, 6.6, 8.0 7.0 11.0	4.6, 7.5, 8.5, 10	6	33
Cod liver oil	19 (18 ♀, 1 ♂)	7 loc. sarc.	all ♀	4.0, 4.0, 4.0, 4.0, 4.6, 4.6, 8.0	7.7, 8.6, 9, 9, 10, 11, 12.5, 12.5	4	37

Notes: [1] Loc. = local, at site of injection.
[2] P. = pellet.
[3] I. = injection.
[4] 1 month = 30 days.
[5] Although this tumor was in close proximity to mammary gland tissue of left axilla, the appearances suggested a very actively growing sarcoma rather than a carcinoma.

The difference between the means of the cod liver oil and tricaprylin groups is significant ($P=0.03$).

DISCUSSION

Contrary to our hypothesis cod liver oil did not show any inhibitory effect on the carcinogenic activity of benzpyrene. This proves that the effect of mouse fat and other "inhibitory" lipid solvents, amongst which we may now also include lecithin and cephalin, is not due to their content of polyethenoid fatty acids. This is in accordance with the findings of Leiter and Shear (8), who showed a correlation between retardation of tumor induction and saturation of the solvent. The abundance of unsaturated fatty acids in cod liver oil may even have been responsible for the earlier appearance of tumors in the group injected with this solvent as compared with the tricaprylin group. A special affinity of growing tumor tissue for highly unsaturated fatty acids is suggested by the observation that these acids disappear from the subcutaneous tissues of rats bearing rapidly growing transplanted tumors (10, 11).

Although the measurements of benzpyrene elimination are open to criticism the results, as far as they go, indicate definite differences in the rate of elimination with the various solvents. If the time of the last measurable observation of benzpyrene is taken as index the elimination of benzpyrene is most rapid with cephalin as solvent, followed by lecithin. The cod liver oil and tricaprylin groups cannot be judged by this standard, but here the calculation of the mean rate of elimination was possible and shows that there is a significantly more rapid disappearance with cod liver oil than with tricaprylin. This difference in elimination is not reflected in any difference of tumor incidence; the shortening of the latent period rather suggests a favorable influence on tumor production by cod liver oil. The lower tumor incidence in the phospholipid group, on the other hand, is also accompanied by a more rapid elimination of benzpyrene.

It might be argued that the greatest weight should be attributed to the lowest K-values found in each group, as they represent presumably those cases where leakage was absent. But even if this is done, the differences of the rates of elimination remain about the same.

The elimination of benzpyrene after subcutaneous injection in sesame oil (0.5 ml.) was measured by Berenblum and Schoental (1). From their data a K of 0.013 can be calculated, which agrees with the lower values found by us for tricaprylin.

TABLE II: DISAPPEARANCE OF 3,4-BENZPYRENE FROM MOUSE AFTER SUBCUTANEOUS APPLICATION

Lecithin			Cephalin			Cod liver oil			Tricaprylin		
Days	S	K	Days	S	K	Days	S	K	Days	S	K
13	126	0.029	0.75	238	0.133	8	(4)	(0.234)	8	220	0.017
18	5.5	0.096	8	(4)	(0.234)	13	61	0.053	13	121	0.030
26	0.9	0.097	13	66.5	0.050	21	52	0.036	21	8.7	0.073
33	0.6	0.082	21	0.4	0.137	28	0.5	0.099	28	18.7	0.043
41	0.3	0.073	28	0.4	0.103	35	5.6	0.049	35	79.5	0.017
47	0.7	0.056	35	<0.3	>0.086	42	<0.3	>0.071	42	18.9	0.029
54	<0.3	>0.055	42	<0.3	>0.086	49	<0.3	>0.061	49	<0.3	>0.061
60	<0.3	>0.055	49	<0.3	>0.086	55	24	0.020	55	15.7	0.023
67	<0.3	>0.055	55	<0.3	>0.086	62	<0.3	>0.048	62	20.2	0.019
74	<0.3	>0.055	62	<0.3	>0.086				69	0.4	0.042
88	<0.3	>0.055	69	<0.3	>0.086				83	7.3	0.019
			83	<0.3	>0.086				97	19.2	0.012
			97	<0.3	>0.086						
Mean = approx. 0.06			Mean = approx. 0.09			Mean = 0.055 Standard deviation = 0.024 Standard error = 0.0083 $\frac{\text{Mean}}{\text{S. E.}} = 6.5$			Mean = 0.032 Standard deviation = 0.019 Standard error = 0.0055 $\frac{\text{Mean}}{\text{S. E.}} = 5.8$		

S=μgm. benzpyrene present at time t.

$K = \frac{1}{t} \log \frac{a}{S}$ (see text).

TABLE III. RECOVERY OF BENZPYRENE 18 HOURS AFTER SUBCUTANEOUS APPLICATION

Lecithin μgm. BP	Cephalin μgm. BP	Cod liver oil μgm. BP	Tricaprylin μgm. BP
241 *	280 (P) *	125 (K)	86 (K)
263 *	258 (P) *	236 (K)	142 (K)
250 *		271 (K)	284 (K)
252 *	238 (I)(K) †	103 (K)	296 (K)
235 *		50 (K)	184 (K)
270 *			155 (K)
172 *			97 (K)
170 *			
233 *			

Notes: BP = benzpyrene.
P = implantation of pellet.
I = injection.
* = postoperative death.
K = killed.

† This value is included in Table II also.

Although the results with lecithin and cephalin are in favor of the assumption that solvent effects are due

to differences of the elimination of benzpyrene, more evidence is clearly necessary before this correlation can be generally established.

SUMMARY

Four groups of mice were given subcutaneous implantations or injections of a single dose of 0.3 mgm. of 3,4-benzpyrene dissolved in (a) purified lecithin; (b) purified cephalin, both from ox brain; (c) medicinal cod liver oil; and (d) tricaprylin. The incidence of local tumors was: lecithin group, 2 of an effective total of 22 (9 per cent); cephalin group, 2 of an effective total of 19 (10.5 per cent); cod liver oil group, 7 of an effective total of 19 (37 per cent); tricaprylin group, 6 of an effective total of 18 (33 per cent). The latent period in the cod liver oil group was approximately 4 months, compared with approximately 6 months for most of the other tumors.

The rate of elimination of benzpyrene was measured and found to differ in the four groups, decreasing in the following order: cephalin, lecithin, cod liver oil, tricaprylin.

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Spontaneous Tumors of the Adrenal Cortex in a Castrated Male Rat

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Spontaneous neoplasms of the adrenal gland in rats are rare. Bullock and Curtis (1) and Curtis, Bullock, and Dunning (2) found four spontaneous tumors of the left adrenal among 31,868 autopsied animals over a number of years. Three of the growths were described as adenomas, and one as a possible cortical cell carcinoma. Ratcliffe (12) found one adrenal adenoma among 237 tumor-bearing rats in a group of over 17,000 animals. Hueper and Martin (10) reported a tumor of the medulla in a castrated male rat fed on a diet deficient in vitamin E. Adrenal tumors are rare in other animals also. Fox (4) mentions among the autopsy findings on 1860 animals (1901-1923) the occurrence of an adrenal adenocarcinoma in a polar bear and a hypernephroma in a California seal. Itami (11) described an adenoma of the left suprarenal in an old female mouse from which an adenocarcinoma in the left axilla had been removed. Haaland (6) reported a spontaneous hypernephroma in the region of the left kidney in a male mouse 22 months old, and Tyzzer (14) two possible hypernephromas in two old female mice. Woolley and his associates (3, 15, 16, 17) found hyperplasia and carcinoma of the adrenal cortex in male and female mice castrated at birth and Gardner (5), also, has reported the occurrence of adrenal tumors in ovariectomized mice. Spiegel (13) found hyperplasia and tumors of the adrenal cortex in guinea pigs castrated at an early age.

The adrenal tumors herein described were observed during routine autopsy on a castrated male rat $\left(\frac{\text{R342}}{\text{20B}}\text{ No. 5}\right)$ 22 months old, weighing 445 gm. The animal was one of a series in which mammary fibroadenoma had been subcutaneously implanted (7), both before and after castration. Four other animals of the same series, weighing between 250 and 340 gm. each, grew large fibroadenomas and had small pale yellow adrenals. Rat 5 did not grow any subcutaneous tumors either before or after castration. At autopsy no trace of the subcutaneous implants was found.

Both adrenals and the pituitary were noticeably enlarged. The left adrenal showed a large cyst, which had displaced or destroyed the medulla and was filled with a finely granular material (Fig. 1). Between cortex and medulla an irregular dark-staining adenomatous tumor surrounded and projected into the cyst. Several smaller cystic areas occurred in the substance of the tumor.

The right adrenal showed a similar adenomatous structure in the juxtamedullary part of the cortex, pushing the medulla to one side (Fig. 2). The tumors, although located centrally, were not new growths of the medulla since the latter was displaced toward the periphery of the gland (Fig. 2), where it was distinctly visible. The cortex showed scattered small cystic areas and evidence of compression (8).

The tumors consisted of compact glands made up of large cuboidal or oval cells (Fig. 3). The cytoplasm was finely granular and the nuclei showed all stages of active mitoses (Fig. 4). There was a close resemblance between these cells and those seen in corpora lutea, and also certain cells in the anterior pituitary gland of castrates. In addition, the pituitary of this rat showed cystic degeneration and numerous castration cells (9).

DISCUSSION

The male rat in which the adrenal cortical adenomas appeared spontaneously had been castrated at 18 months of age and was resistant to tumor transplantation before and after castration. Castrated males of the same series that grew transplanted benign subcutaneous tumors showed normal but involuted adrenals.

No conclusion can be drawn from the appearance of adrenal adenomata in a castrated male resistant to the growth of implanted mammary tumors. However, further investigations are advisable in old animals, as all other reported adrenal tumors appeared in old animals.

CONCLUSION

An adrenal cortical adenoma is described in an old castrated male rat resistant to subcutaneously implanted mammary fibroadenoma.

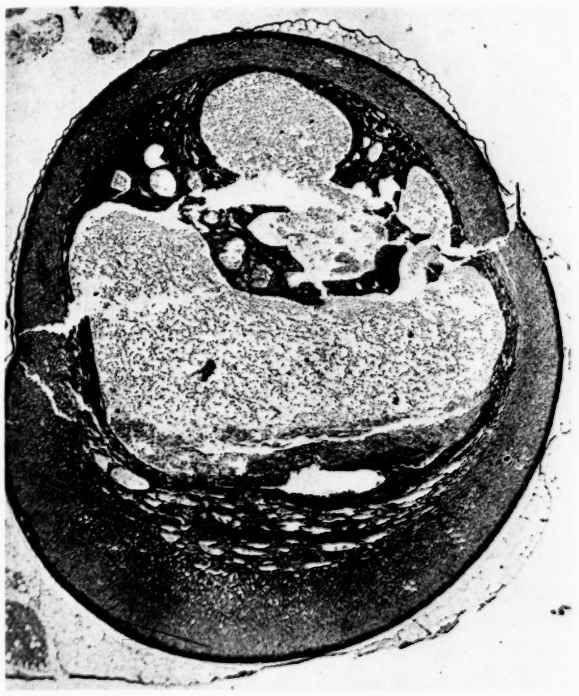

FIG. 1.—Left adrenal gland in an old castrated male rat. Adenoma with cystic degeneration, in juxtamedullary zone. × 48.

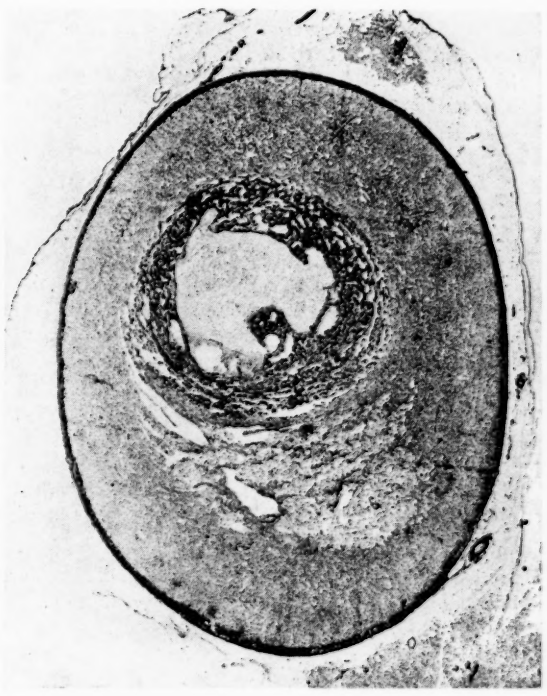

FIG. 2.—Right adrenal in same rat. Adenoma replacing juxtamedullary zone. Medulla pushed aside, between tumor and cortex. × 48.

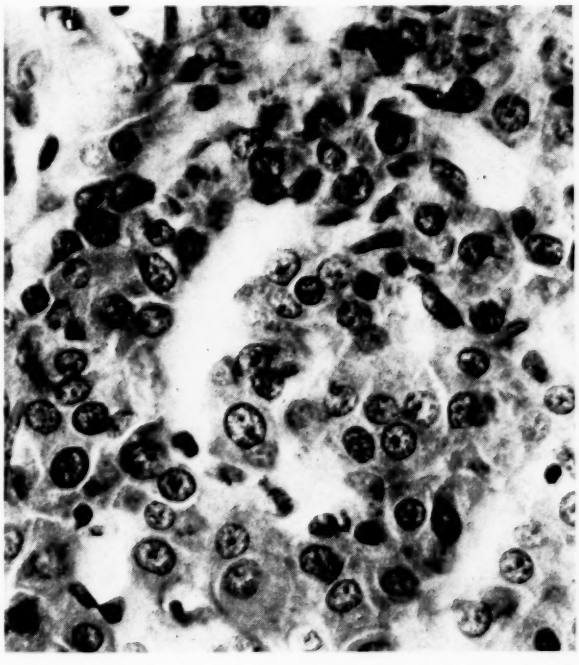

FIG. 3.—Adenoma of rat adrenal. Large cells with granular cytoplasm, dark-staining nuclei. × 300.

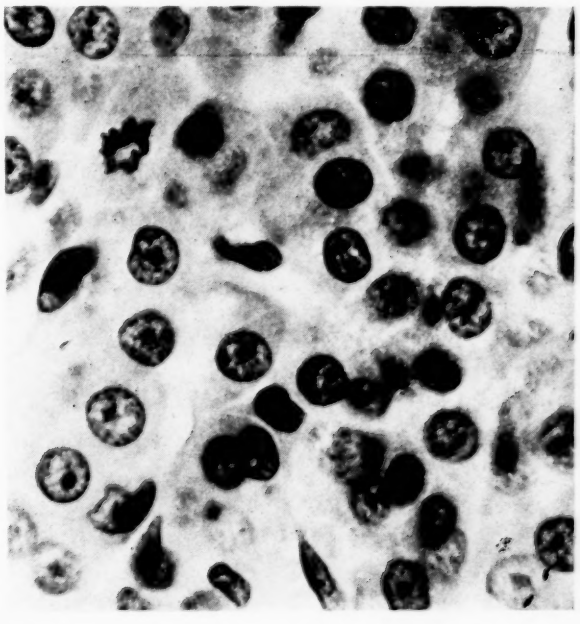

FIG. 4.—Higher magnification. Nuclei show active mitoses. × 600.

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The Racial Distribution of Cancer

I. Epithelial Tumors of the Skin, Lip, and Breast*

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In preparing an annual report on the activities of the tumor clinic of Hines Hospital (14) it was observed that some types of cancer occurred almost exclusively in white patients whereas other forms occurred with relative frequency in colored men. To determine the significance of these observations a study was made of the racial distribution of 13,489 male patients who were admitted to this hospital during 1931 through 1942 and whose lesions were diagnosed either as carcinomatous or benign. The findings were checked as far as possible by an analysis of the Mortality Statistics of the United States during 1930 through 1934 (10) and of the data obtained by the U. S. Public Health Service on the prevalence of cancer in selected areas of the United States (3, 7, 9, 11, 15, and 16).

The purpose of this work is the collection of statistics on the incidence of cancer in the white and colored races. An attempt is made also to determine whether the observed differences in racial distribution of certain types of tumor are statistically and biologically significant, and whether they are due to intrinsic racial differences in susceptibility to cancer or to extrinsic environmental factors.

Statistical Methods

A statistic which might be called the "percentage colored" was determined for each type of tumor. This term is defined as 100 times the ratio of colored patients to all patients with a particular neoplasm.

Table I: Method for Determining the Control Group for Male Patients with Cancer in Hines Hospital

Tumor (1)	No. of patients, all races (2)	No. of colored patients (3)	Percentage colored (4)	Trial control group *			Difference between percentage colored for trial control group and that for all tumors (8)
				No. of patients, all races (5)	No. of colored patients (6)	Percentage colored (7)	
All cancerous tumors	11,790	742	6.30				
Carcinoma of:							
Lip	1,207	6	0.50	10,583	736	6.95	+ 0.66
Exposed skin	2,011	12	0.60	9,779	730	7.46	+ 1.17
Testis	252	5	1.98	11,538	737	6.39	+ 0.10
Melanoma of:							
Skin	151	6	3.97	11,639	736	6.32	+ 0.03
Carcinoma of:							
Sigmoid & rectum	753	55	7.30	11,037	687	6.22	— 0.07
Stomach	648	70	10.80	11,142	672	6.03	— 0.26
Prostate	367	42	11.44	11,423	700	6.13	— 0.16
Esophagus	227	41	18.06	11,563	701	6.06	— 0.23
Penis	118	33	27.97	11,672	709	6.07	— 0.22

* A trial control group consists of all patients with cancer except those with one particular type of tumor.

To determine whether the percentage colored for a tumor was high or low it was compared with that for a control group which consisted of all patients with cancer except those with certain types of growth. It was observed that the percentage colored for all tumors was inordinately affected by two or three types of tumors which affected large numbers of individuals and which had unusual racial distributions. It was decided to exclude patients with these neoplasms from the control group.

In Table I are given the preliminary calculations needed to determine the control group for male patients with cancer in Hines Hospital. This table shows the percentage colored for all tumors and for a few neoplasms that affected a large number of patients

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or that had a very low or high percentage colored. Trial control groups were obtained by subtracting the number of individuals with each cancer from the number of patients with all tumors. The differences in the percentage colored for the trial control groups and for all tumors were calculated, and are shown in column 8 in the table. It is seen from this column that the only tumors that gave large differences (more than 0.5 per cent) were carcinoma of the exposed skin and of the lip. Accordingly, the control group in Hines Hospital was defined as all male patients with cancer, except those with carcinoma of the exposed skin and lip. For convenience and consistency the tumors of the skin and lip were also excluded from the control groups for men and women in the analysis of the data from the Public Health Service and the Mortality Statistics of the United States.

The difference between the percentage colored of each tumor studied and that for the control group was then obtained. In the preliminary investigations the statistical significance of the difference in percentages was obtained by means of the nomogram described by Schrek (13). In final studies the standard error of the difference was calculated by means of Yule's formula (17), which may be rewritten as:

$$s^2 = \frac{10{,}000\,p(100-p)}{d^2}\left(\frac{1}{n}-\frac{1}{N}\right)$$

where d is the difference in percentages, p is the percentage colored in the control group, N is the number of persons in the control group, n is the number of individuals with a particular type of tumor, and s is the standard error of the difference expressed as a percentage of the difference. If the tumor studied is one that is excluded from the control group, it is necessary to use another formula (17):

$$s^2 = \frac{10{,}000\,P(100-P)}{d^2}\left(\frac{1}{n}+\frac{1}{N}\right)$$

where P is the percentage colored in the combined groups $(N+n)$. The difference was considered significant when the error was low ($s = 38.8$ per cent or less) and not significant when high (51.0 per cent or more). When the error was between 38.8 and 51.0 per cent the significance was doubtful, and additional statistical tests were performed if possible.

The data collected by the Public Health Service also were analyzed by the methods described above. The Public Health Service determined the number of men and women with cancer in several geographic areas in the United States. The percentage colored for each tumor and for the control groups in each of five areas was calculated. The Mortality Statistics of the United States afforded additional material for the study of racial distribution of cancer. The actual numbers of deaths for each type of cancer and for each age group were obtained for the years 1930 through 1934.

Of the three sources of data utilized in this study the Mortality Statistics are the least accurate, because of diagnostic errors particularly of tumors of the internal organs. It is quite definite that these errors were more common among the colored than the white population. For example, in 1934 there were 13,797 deaths listed as "Cause of death not specified or unknown." Of these fully 50.7 per cent were colored. There is probably an underestimation of the number of colored persons with cancer of internal organs such as the kidney, stomach, and brain.

A Comparison of the Control Groups

For the male patients of Hines Hospital separate control groups were set up for patients with cancer and for those with benign tumors and related lesions. It is seen from Table II that the percentages colored for the two control groups for all years were approximately the same (8.45 percentage colored for cancer and 8.50 for benign lesions).

According to the data of the Public Health Service and the Mortality Statistics, the percentage colored of the female control groups was slightly but significantly higher than that for the male control groups (6.83 per cent for women as compared to 5.61 per cent for men in the Public Health survey; 5.27 per cent and 4.75 per cent respectively for the Mortality Statistics). Differences in the percentages colored for the male and female control groups were consistently less than the differences in the percentages colored for male and female patients with all tumors.

The validity of the use of the control groups is indicated by the equality of the percentage colored for cancer and for benign tumors in Hines Hospital, and by the smallness of the difference of the percentage colored for male and female control groups.

Carcinoma of the Skin

In view of the theory that direct exposure to sunlight is an important etiologic factor in cancer of the skin, Schrek (12) differentiated between carcinoma of the skin exposed to the elements (face, ears, neck, hands, and wrists) and carcinoma of the covered skin (scalp, trunk, arms, legs, and feet).

Carcinoma of the exposed skin was the most frequently observed tumor in Hines Hospital (17.1 per cent of all patients with cancer). It had a very low percentage colored (only 0.6 per cent), which was significantly less than that for the control group (8.45 per cent). In contrast, carcinoma of the covered skin was relatively rare (0.8 per cent of all patients) but had a fairly high percentage colored (9.78 per cent), which did not differ appreciably from that for the control group. The statistics for carcinoma of the covered and exposed skin are, then, quite distinct.

The data presented in the Public Health Reports and in Mortality Statistics do not differentiate between tumors of the exposed and covered skin or between carcinoma and other neoplasms. Inasmuch as malignant tumors other than carcinoma of the exposed skin are relatively uncommon, it is permissible to compare the published data on cancer of the skin with the present findings on carcinoma of the exposed skin.

The reports of the Public Health Service show that tumors of the skin are common both in men and women (20.9 per cent of male and 11.0 per cent of female patients with cancer). The percentages colored were significantly low in both sexes (0.89 and 1.91 per cent). The data of Hines Hospital on carcinoma of the exposed skin and those of the U. S. Public Health Service on tumors of the skin are in good agreement as to both the frequency of the disease (17.1 per cent and 20.9 per cent) and its racial distribution (0.60 and 0.89 percentage colored).

The Mortality Statistics reported relatively few deaths with tumor of the skin (3.5 per cent of all deaths with cancer in men and 1.7 per cent in women). Dorn and Horn (4) discussed in detail the reasons for the low number of recorded deaths with tumor of the skin. The percentages colored for this neoplasm were 2.07 for men and 3.83 for women. These percentages are significantly lower than those for the control groups (4.75 and 5.27 per cent respectively).

It may be concluded that carcinoma of the exposed skin occurs with relative infrequency in colored patients, whereas carcinoma of the covered skin has the same racial distribution as in the control group.

Carcinoma of the Lip

According to the statistics of Hines Hospital and of the Public Health Service cancer of the lip is common among men, but not in women (10.2 and 7.3 per cent of all male patients and 0.5 per cent of female patients). Cancer of the lip, like that of the exposed skin, occurred in relatively few colored persons (0.50 and 0.62 percentage colored for male patients, 1.70 percentage colored for women).

Table II: The Racial Distribution of Tumors of the Skin, Lip, and Breast According to Data from Three Sources

	Percentage colored					Number of patients, all races				
	Male			Female		Male			Female	
	Hines Hospital, 1931-42	U. S. Public Health Survey	U. S. Mortality Statistics, 1930-34	U. S. Public Health Survey	U. S. Mortality Statistics	Hines Hospital	U. S. Public Health Survey	U. S. Mortality Statistics	U. S. Public Health Survey	U. S. Mortality Statistics
Cancer, all types	6.29	4.24	4.61	8.32	7.00	11,790	19,615	278,860	24,735	336,844
Benign lesions, all types	5.82					1,699				
Control groups										
Cancer	8.45 ‡	5.61 §	4.75 §	6.83 †	5.27 †	8,572	14,022	265,993	15,100	256,945
Benign tumors	8.50 ‖					1,106				
Skin, cancer		0.89 *	2.07 *	1.91 *	3.83 *		4,103	9,872	2,717	5,834
Exposed skin, carcinoma	0.60 *					2,011				
Covered skin, carcinoma	9.78					92				
Lip, cancer		0.62 *	0.86 *	1.70	4.92		1,440	2,995	117	284
Lip, carcinoma	0.50 *					1,207				
Breast, cancer		14.0 *	7.76 *	6.88	5.98 *		49	811	6,722	58,823
Breast, carcinoma	26.0 *					23				
Skin, keratosis	1.11 *					449				

* The difference between this percentage colored and that of the corresponding control group is statistically significant.
† The difference between this percentage colored and that of the control group for men is statistically significant.

Composition of control groups:
‡ All male patients with cancer except those with carcinoma of the exposed skin and lip.
§ All men with cancer except those with cancer of the skin and lip.
‖ All male patients with benign lesions except those with keratosis and leukoplakia.
¶ All women with cancer except those with cancer of the skin, lip, uterus, and cervix.

Keratosis of the Skin

The most common type of benign lesion observed in Hines Hospital was keratosis of the skin (26.4 per cent of all men treated for benign tumors or related lesions). Only those cases that were not associated with cutaneous carcinoma are considered in this analysis. The keratosis occurred almost exclusively on the exposed skin. This lesion had a percentage colored significantly lower than that for the control group (1.11 as compared to 8.50 per cent for the control). It is of interest that keratosis of the skin and carcinoma of the exposed skin and of the lip had approximately the same racial distribution (1.11, 0.60, and 0.50 per cent colored respectively).

Carcinoma of the Breast

The data of both the Public Health Service and the Mortality Statistics indicate that cancer of the breast in women is common (27.2 per cent and 17.5 per cent of female cancerous patients). The Mortality Statistics show that this tumor has a percentage colored (5.98 per cent) slightly but significantly higher than that for the control group (5.27 per cent). This significant difference in percentages colored was not observed in the statistics of the Public Health Service (6.88 and 6.83 per cent), possibly as a result of the smaller number of cases in the survey of the Public Health Service.

In contrast to cancer of the breast in women, this tumor in the male is very rare (0.20, 0.25, and 0.29 per cent of all men with cancer, according to the data of Hines Hospital, the Public Health Service, and Mortality Statistics). The three sources of data agree in the finding that the percentage colored for this tumor in men is considerably and significantly higher than those for the control group (26, 14, and 7.76 percentage colored for tumor of the male breast, as compared to 8.45, 5.61, and 4.75 per cent for the control groups).

It is concluded that cancer of the male breast is rare, but occurs with relatively greater frequency in colored patients. In contrast, tumor of the female breast is common and this growth has probably a slightly higher incidence in colored than in white women.

DISCUSSION

Much work has been done on the relative incidence of cancer in general in the white, colored, and other races. These studies have been reviewed by Lewis (6). According to Cheatle & Cutler (1) the various races have the same susceptibility to cancer in general, but show notable differences in the incidence for different organs. If one considers Ewing's dictum that cancer is not a single disease but a large group of diseases, it would seem important to study the racial distribution of each type of tumor.

The customary procedure in studying racial distribution is the determination of the incidence or death rates for a particular tumor in a known population; for example, Dublin (5) determined the death rates for various types of tumor in Metropolitan policyholders. But in studying the patients of a tumor clinic it is usually difficult or impossible to determine the size or the constitution of the population from which the patients are drawn, and incidence or death rates therefore cannot be calculated.

The method used in this study consists in determining what percentage of patients with a given tumor are colored. This percentage is compared with that obtained for a control group which consists of all patients with tumor except those with certain types of neoplasms.

If the difference between the percentage colored of a tumor and that of the control group is statistically significant, one has to consider whether the difference is due to fortuitous or to biological factors.

One of the chief fortuitous factors that may lead to misleading findings on racial distribution is non-random selection of cases. The effect of this disturbing factor can be partially evaluated by a careful and detailed appraisal of the type of patients admitted to a tumor clinic. A second fortuitous factor is non-random errors of diagnosis. If the errors are just as likely to occur in the patients of one race as in those of another, they will tend to obscure real differences in racial distribution and will not lead to misleading differences. If the errors are more common in one race, they may lead to differences in percentages colored that are statistically but not biologically significant. These two fortuitous factors are difficult to eliminate in data from a single source, and a comparison of the results from several diverse sources aids to a considerable extent in minimizing the influence of non-random selection of cases and errors of diagnosis.

The biological factors that may be responsible for differences in racial distribution are inherent racial differences in susceptibility or resistance to a particular type of tumor and external, or environmental, factors. The racial differences may be the result of: (a) the presence or absence of some unknown intracellular factor, and (b) the relative presence or absence of extracellular factors such as hormones in the blood or pigment in the skin. The intrinsic intracellular factor is called by Cramer (2) the proximate cause, and the environmental factors are called the remote causes of cancer.

Carcinoma and Keratosis of the Skin and Lip

It is well established that carcinoma and keratosis of the skin and lip are relatively uncommon in colored persons. This finding is usually attributed to the cutaneous pigment of negroes, which protects the epithelial cells from the carcinogenic effects of sunlight. The present finding that only carcinoma of the exposed skin, but not that of the covered skin, is associated with a low percentage colored adds additional evidence that sunlight is the principal etiologic agent in carcinoma of the exposed skin. The low percentage colored for carcinoma of the lip suggests that, in the United States at least, cancer of the lip is largely the result of exposure to sunlight. It is concluded that the low percentage colored for carcinoma and keratosis of the exposed skin and lip is the result of an extracel-

lular factor; namely, the varying amount of cutaneous pigment in the white and colored races.

Carcinoma of the covered skin, on the other hand, has a percentage colored that is slightly greater than that for the control group. A larger group of cases would be needed to determine whether the small difference in the percentages colored is statistically significant. The finding of a "normal" or high percentage colored for carcinoma of the covered skin suggests that there is no inherent cellular resistance or immunity of the epithelial cells of the negro to cancer. Previous studies (12) have shown that important etiologic agents for this tumor are pre-existing chronic inflammatory lesions and scars. These factors in colored men are presumably as common as, or more common than, in white men.

Cancer of the Breast

According to the data of Hines Hospital, the Public Health Service, and Mortality Statistics, carcinoma of the breast in the male has a considerably higher percentage colored than the control group. It seems quite definite that this tumor, although rare, is much more common in colored than in white men.

Carcinoma of the breast in women had approximately the same percentage colored as that of the control group according to the data of the U. S. Public Health Service. Analysis of the data in Mortality Statistics, however, showed small but significant differences. As Mortality Statistics is based on very large numbers of cases it may reveal differences that are too small to be observed in the data of the U. S. Public Health Service, and inasmuch as the tumor is in an accessible site, and errors of diagnosis are not unduly large, the results of Mortality Statistics may be accepted. Thus it is probable that cancer of the breast occurs with relatively greater frequency in colored than in white women.

It is not possible to state at the present time what are the biologic factors responsible for the high percentage colored in cancer of the breast in men and the slightly elevated percentage in women. Strains of mice differ considerably in the incidence of breast cancer as a result of selective breeding, and it may be that races of men differ in the incidence of this tumor as a result of intrinsic racial susceptibility.

SUMMARY

The racial distribution of cancer was studied by analysis of data from (a) the records of Edward Hines, Jr., Memorial Hospital, (b) the U. S. Public Health Survey on the prevalence of cancer, and (c) the Mortality Statistics of the United States.

Carcinoma of the exposed skin and of the lip, and keratosis of the skin, had very low percentage colored. Carcinoma of the covered skin, however, had approximately the same percentage colored as the control group.

Cancer of the breast in the male had a very high percentage colored. The percentage colored for tumor of the breast in the female was slightly elevated.

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The Clinical Effects of Aldehyde Bisulfites in Patients with Cancer

I. The Administration of Heptylaldehyde Bisulfite to Patients with Inoperable Mammary Carcinoma Metastatic to Bone

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The oral administration of heptylaldehyde bisulfite [1] to mice bearing spontaneous mammary carcinoma is reported to produce irregularly a liquefaction necrosis of the tumors (4, 8). There is no agreement that the amounts of the compound fed induce systemic reactions. Recent experiments in this Hospital indicate that heptylaldehyde bisulfite is moderately toxic for human breast carcinoma *in vitro* also (5). For these reasons the material was given to patients with that disorder. The results form the subject of the present communication.

MATERIAL AND METHODS

The heptylaldehyde bisulfite was administered to 14 women of from 31 to 61 years of age. All were known to have had carcinoma of the breast for from 1 to 15 years. The diagnosis was made in all by biopsy of the primary lesion if inoperable or from a review of a previous section if recurrent. In 4 instances the primary lesion was inoperable; in the others it had recurred after operation. Roentgenograms revealed skeletal lesions in all the patients consistent with a diagnosis of metastases to the bones. During the study 3 patients were hospitalized and the remainder were seen in the clinic every 2 weeks. None received x-radiation therapy at any time during the administration of the drug.

The heptylaldehyde bisulfite was given to the 3 hospitalized patients by a continuous intravenous drip for from 14 to 21 days. The amount administered daily by this means was about 0.5 gm. per kilogram body weight. The patients occasionally manifested evidence of a toxic effect, indicated by diarrhea, headaches, and small convulsive movements. When the amount of compound administered was increased to about 0.55 gm. per kilogram daily, these toxic reactions were severe.

[1] Sodium alpha hydroxy heptane sulfonate; this compound was generously supplied by the Calco Chemical Division of the American Cyanamid Company.

Each patient seen in the clinic received daily from 1 to 12 gm. of the heptylaldehyde bisulfite. These amounts were administered in 3 divided doses at meal time. Pyrosis and nausea sometimes occurred if no food was taken with the drug.

In the course of the investigation measurements were made of the serum calcium by the technic of Clark and Collip (3); of the serum inorganic phosphorous by the method of Kuttner and Lichtenstein (7); of the serum alkaline phosphatase by the procedure of Bodansky (2) as modified by Woodward (10); and of the blood urea nitrogen by the method of Van Slyke and Cullen (9).

The functional capacity of the liver was estimated from measurements of serum bilirubin, protein, cholesterol and cholesterol esters, plasma prothrombin, and mean erythrocyte volume. The methods, and the evidence indicating that the tests employed provide indices of hepatic function, have been presented in a previous communication (1). Blood counts, urine analyses, and roentgenograms of the bones were obtained by routine technics.

RESULTS

A. The Daily Oral Administration of 1 gm. of Heptylaldehyde Bisulfite

Three patients received orally 1 gm. of heptylaldehyde bisulfite each day for from 20 to 67 days (Table I). In the course of treatment, 1 patient observed no symptomatic change, the second had considerable relief from long-continued nausea, and the third reported a decrease of bone pain. The symptomatic improvement of the last patient, however, was associated with x-ray evidence of a widespread increase in skeletal metastases. This was not the case in the other 2 patients. No obvious changes occurred in size or consistency of the local breast lesion in the 1 patient (G.M.) who still bore the primary tumor.

The concentrations of calcium and inorganic phos-

phorus in the serum of the patients studied were not altered significantly. In 1 instance (G.M.) the serum alkaline phosphatase decreased from 22.8 to 3.0 units, but this change followed the removal of considerable ascitic fluid, which by compression may have decreased the ability of the liver to excrete the phosphatase into the bile (Table II).

155 to 231 days (Table V). During the course of therapy the pain referable to the skeletal metastases remained unchanged in 1 instance, became progressively worse in 3, and decreased slightly in another. After the compound had been administered to this last patient for 231 days, x-ray examination of the skeleton revealed further spread of metastases. Roentgenograms indicated a similar progression of metastases in all but 1 of the remaining 4 patients. There was no change in the size or consistency of the local breast lesions in the 3 instances where the primary lesion still was present.

TABLE I: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 1 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Age	Breast carcinoma discovered in	Radical mastectomy performed in	Skeletal metastases discovered in	Days treated	Effect on primary lesion	Effect on symptoms	Effect on skeletal metastases (in roentgenograms)
A.G.	38	July, 1941	July, 1941	June, 1942	20	....	None	None
G.M.	45	Aug., 1942	Inoperable	Sept., 1942	67	None	Decreased nausea	None
A.E.	42	June, 1940	Sept., 1940	June, 1942	38	....	Definite relief from bone pain	Definite increase in size and number

TABLE II: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 1 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Serum calcium, mgm. per cent		Serum inorganic phosphorus, mgm. per cent		Serum alkaline phosphatase, units per cent	
		Before	After	Before	After	Before	After
A.G.	20	16.6	16.6	5.1	4.4	14.1	16.3
G.M.	67	9.5	10.3	3.4	3.8	22.8	3.0
A.E.	38	11.3	11.0	3.5	4.5	4.2	4.5

TABLE III: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 1 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	R. B. C., million per mm.^{3}		Hb., per cent		W. B. C. per mm.^{3}		Polynuclear cells, per cent		Blood urea N, mgm. per cent		Urine	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
A.G.	20	3.66	3.16	65	60	7,150	7,500	69	77	28.0	18.0	Neg.	Neg.
G.M.	67	3.70	3.44	71	69	3,700	6,100	65	68	8.3	14.3	"	"
A.E.	38	3.68	3.78	75	77	8,800	10,400	87	77	16.4	13.0	"	"

TABLE IV: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 1 GM. OF HEPTYLALDEHYDE BISULFITE ON THE HEPATIC FUNCTIONS OF PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Mean erythrocyte volume, μ^3		Plasma prothrombin, per cent		Serum bilirubin, mgm. per cent		Serum cholesterol and cholesterol esters, mgm. per cent		Serum protein, gm. per cent	
		Before	After	Before	After	Before	After	Before	After	Before	After
A.G.	20	79	81	90	100	1.25	1.3	63/126	75/152	6.8	6.5
G.M.	67	89	89	100	96	1.25	2.1	68/128	101/147	4.5	5.8
A.E.	38	90	91	100	86	1.15	1.8	57/125	59/153	7.7	7.3

No patient developed anemia, leukopenia, agranulocytosis, or evidence of hepatic dysfunction. The urine of all remained free of red cells, albumin, and casts, and the blood urea nitrogen levels did not increase beyond normal limits (Table III and IV).

B. THE DAILY ORAL ADMINISTRATION OF 6 GM. OF HEPTYLALDEHYDE BISULFITE

Four patients, and 1 of the 3 who formerly had received 1 gm. of heptylaldehyde bisulfite (G.M.), were given 6 gm. of the compound each day for from

The concentrations of calcium and inorganic phosphorus in the serum of these 5 patients were not altered significantly during the period of observation. In 1 instance the serum alkaline phosphatase level increased distinctly (Table VI). The red cell count and hemoglobin concentration decreased in 3 patients,

TABLE V: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 6 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Age	Breast carcinoma discovered in	Radical mastectomy performed in	Skeletal metastases discovered in	Days treated	Effect on primary lesion	Effect on symptoms	Effect on skeletal metastases (in roentgenograms)
A.K.	55	Apr., 1936	Apr., 1936	Feb., 1943	155	....	None	None
A.P.	50	July, 1940	Inoperable	July, 1940	204	None	Mild decrease of bone pain	Further spread of bone metastases
R.F.	47	Feb., 1942	Inoperable	Feb., 1942	231	None	Mild increase of bone pain	Further spread of bone metasteses
F.K.	48	Jan., 1939	Jan., 1939	Apr., 1941	196	....	Considerable increase of bone pain	Further spread of bone metastases
G.M.	45	Aug., 1942	Inoperable	Sept., 1942	198	None	Considerable increase of bone pain	Further spread of bone metastases

TABLE VI: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 6 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Serum calcium, mgm. per cent		Serum inorganic phosphorus, mgm. per cent		Serum alkaline phosphatase, mgm. per cent	
		Before	After	Before	After	Before	After
A.K.	155	11.6	11.7	4.5	3.8	6.3	11.8
A.P.	204	10.8	10.5	4.2	4.5	8.2	8.7
R.F.	231	10.8	11.0	4.5	3.7	7.2	3.7
F.K.	196	12.0	13.0	4.5	4.7	8.0	8.5
G.M.	198	10.3	10.2	3.8	4.3	3.0	6.2

TABLE VII: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 6 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	R. B. C., million per mm.^{3}		Hb., per cent		W. B. C. per mm.^{3}		Polynuclear cells, per cent		Blood urea N, mgm. per cent		Urine		Weight	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
A.K.	155	4.01	3.20	78	60	4,950	7,800	79	68	14.6	13.6	Neg.	Neg.	141	130
A.P.	204	2.99	2.92	59	50	5,600	5,650	80	76	9.8	10.6	"	"	180	180
R.F.	231	3.36	3.53	69	70	6,100	4,700	60	71	13.2	11.5	"	"	162	163
F.K.	196	3.28	2.92	68	58	5,700	7,400	76	70	10.9	8.7	"	"	97	85
G.M.	198	3.44	3.02	69	55	6,100	5,500	68	80	14.3	10.9	"	"	ascites	

TABLE VIII: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 6 GM. OF HEPTYLALDEHYDE BISULFITE ON THE HEPATIC FUNCTIONS OF PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Mean erythrocyte volume, μ^3		Plasma prothrombin, per cent		Serum bilirubin, mgm. per cent		Serum cholesterol and cholesterol esters, mgm. per cent		Serum protein, gm. per cent	
		Before	After	Before	After	Before	After	Before	After	Before	After
A.K.	155	87	99	67	67	0.9	0.8	50/112	62/129	7.0	7.1
A.P.	204	93	90	87	76	1.8	1.0	63/141	52/144	7.2	6.9
R.F.	231	100	96	65	68	1.0	1.0	73/53	66/155	7.0	7.7
F.K.	196	98	100	100	74	0.5	0.5	54/118	68/139	7.3	6.9
G.M.	198	89	98	96	72	2.1	1.6	101/147	64/134	4.9	5.8

but none developed leukopenia or agranulocytosis. In all 5 the blood urea nitrogen concentrations remained within normal limits and the urine free of albumin, red cells, and casts (Table VII). No evidence of progressive hepatic insufficiency was discovered (Table VIII). At the end of the period of treatment, 3 patients had lost considerable weight and were considered terminal.

C. THE DAILY ORAL ADMINISTRATION OF 12 GM. OF HEPTYLALDEHYDE BISULFITE

The amount of this material found to be most effective in the treatment of mammary carcinoma in mice was 6 mgm. daily (8). On a comparative weight basis, this optimum daily dose corresponds to about 12 gm. for a 50 kilogram woman. Accordingly, 12 gm. of the compound was administered daily to each

of 4 patients for from 31 to 78 days. None experienced any decrease of weakness or of pain referable to skeletal metastases. Roentgenograms failed to reveal any regression of the metastatic process in 2 instances, and further spread in the remainder.

peared desirable to maintain constantly as high a concentration of the compound as possible. Hence 3 patients were given increasing amounts of heptylaldehyde bisulfite by continuous intravenous drip until 0.55 gm. per kilogram body weight were given daily. At this

TABLE IX: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 12 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Age	Breast carcinoma discovered in	Radical mastectomy performed in	Skeletal metastases discovered in	Days treated	Effect on primary lesion	Effect on symptoms	Effect on skeletal metastases (in roentgenograms)
T.M.	49	Sept., 1934	Sept., 1934	May, 1943	78	....	None	Increase in size and number
M.D.	43	Jan., 1942	Jan., 1942	Aug., 1943	31	....	None	Increase in size and number
M.Z.	58	Oct., 1935	Oct., 1935	Aug., 1943	56	....	None	No change
R.O.	45	July, 1928	Apr., 1930	Feb., 1943	56	....	Increase in severity	No change

TABLE X: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 12 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Serum calcium, mgm. per cent		Serum inorganic phosphorus, mgm. per cent		Serum alkaline phosphatase, units per cent	
		Before	After	Before	After	Before	After
T.M.	78	11.2	11.0	4.7	4.0	4.0	4.1
M.D.	31	11.6	10.6	3.6	4.4	2.2	3.6
M.Z.	56	11.2	11.0	3.8	3.8	4.7	5.6
R.O.	56	11.2	10.8	3.3	4.6	4.3	3.1

TABLE XI: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 12 GM. OF HEPTYLALDEHYDE BISULFITE TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	R. B. C., million per mm.^{3}		Hb., per cent		W. B. C. per mm.^{3}		Polynuclear cells, per cent		Blood urea N, mgm. per cent		Urine		Weight	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
T.M.	78	3.74	3.11	71	65	5,500	6,800	61	70	9.9	12.7	Neg.	Neg.	114	110
M.D.	31	3.84	3.56	80	74	11,200	8,800	85	71	13.9	11.1	"	"	126	118
M.Z.	56	3.85	4.14	76	80	11,400	14,400	66	78	11.7	12.4	"	"	166	155
R.O.	56	2.58	2.47	61	52	8,100	7,400	72	80	7.2	14.0	"	"	134	116

TABLE XII: THE EFFECTS OF THE DAILY ORAL ADMINISTRATION OF 12 GM. OF HEPTYLALDEHYDE BISULFITE ON THE HEPATIC FUNCTIONS OF PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Mean erythrocyte volume, μ^3		Plasma prothrombin, per cent		Serum bilirubin, mgm. per cent		Serum cholesterol and cholesterol esters, mgm. per cent		Serum protein, gm. per cent	
		Before	After	Before	After	Before	After	Before	After	Before	After
T.M.	78	93	90	69	75	1.5	1.2	62/127	60/135	7.4	6.8
M.D.	31	85	87	94	100	1.3	0.6	48/103	40/145	6.7	7.1
M.Z.	56	88	92	84	90	1.0	1.0	81/156	.....	5.9	6.1
R.O.	56	80	92	72	76	0.8	1.2	59/126	45/121	...	...

As in the previous groups of patients, no significant effect was observed on the serum concentrations of calcium, phosphorus, or phosphatase, and, by the criteria used, on the hemograms or on the hepatic and renal functions.

D. The Continuous Intravenous Administration of Heptylaldehyde Bisulfite

Since the material had failed to influence the course of the disease in the patients thus far studied, it appeared desirable to maintain constantly as high a concentration of the compound as possible. Hence 3 patients were given increasing amounts of heptylaldehyde bisulfite by continuous intravenous drip until 0.55 gm. per kilogram body weight were given daily. At this level each developed nausea, diarrhea, occasional headaches, and small clonic convulsive movements, so that it was necessary to reduce the amount administered to 0.50 gm. per kilogram daily.

After this quantity had been given for from 14 to 21 days, no effect was noted in the extent or microscopic appearance of the primary lesion of the 1 patient in whom this still was present (J.B.). Roentgenograms indicated a further continued spread of skeletal metastases in all, and in only 1 instance did the pain refer-

able to these metastases decrease. In none did the serum concentrations of calcium, phosphorus, or alkaline phosphatase change considerably, and no evidence of significant renal, hepatic, or hematopoietic damage could be found by the methods used. Four weeks after the medication was discontinued, 2 of these 3 patients were considered to be terminal.

failure was not altogether unexpected, for the following reasons:

(a) From studies now under way in this Hospital, the inhibition of tissue respiration exerted by the compound *in vitro* is due to its bisulfite portion (6).

(b) When 12 gm. of heptylaldehyde bisulfite were fed to 2 patients in a fasting state, no sulfite could

TABLE XIII: THE EFFECTS OF THE CONTINUOUS INTRAVENOUS ADMINISTRATION OF HEPTYLALDEHYDE BISULFITE (0.5 GM. PER KILOGRAM DAILY) TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Age	Breast carcinoma discovered in	Radical mastectomy performed in	Skeletal metastases discovered in	Days treated	Effect on primary lesion	Effect on symptoms	Effect on skeletal metastases (in roentgenograms)
C.B.	31	Jan., 1943	Mar., 1943	Sept., 1943	14	.....	None	Definite increase in size and number
J.B.	61	Sept., 1943	Inoperable	Sept., 1943	21	Increased in size; no change in microscopic appearance	None	Increased areas of bone destruction
A.V.	46	Mar., 1940	Apr., 1940	July, 1943	20	.....	Moderate remission	Increase in size and number

TABLE XIV: THE EFFECTS OF THE CONTINUOUS INTRAVENOUS ADMINISTRATION OF HEPTYLALDEHYDE BISULFITE (0.5 GM. PER KILOGRAM DAILY) TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Serum calcium, mgm. per cent		Serum inorganic phosphorus, mgm. per cent		Serum alkaline phosphatase, units per cent	
		Before	After	Before	After	Before	After
C.B.	14	11.8	11.5	4.3	5.6	5.4	7.9
J.B.	21	11.4	11.8	4.9	4.4	3.7	3.2
A.V.	20	11.1	10.6	4.7	4.4	3.9	2.6

TABLE XV: THE EFFECTS OF THE CONTINUOUS INTRAVENOUS ADMINISTRATION OF HEPTYLALDEHYDE BISULFITE (0.5 GM. PER KILOGRAM DAILY) TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	R. B. C., million per mm³		Hb., per cent		W. B. C. per mm³		Polynuclear cells, per cent		Blood urea N, mgm. per cent		Urine	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
C.B.	14	3.70	3.15	65	62	7,150	8,800	80	90	9.4	11.1	Neg.	Neg.
J.B.	21	2.90	3.20	56	60	6,200	4,200	62	61	11.0	7.0	"	"
A.V.	20	3.49	3.57	65	60	7,300	5,500	60	70	10.0	9.3	"	"

TABLE XVI: THE EFFECTS OF THE CONTINUOUS INTRAVENOUS ADMINISTRATION OF HEPTYLALDEHYDE BISULFITE (0.5 GM. PER KILOGRAM DAILY) TO PATIENTS WITH CARCINOMA OF THE BREAST METASTATIC TO BONE

Patient	Days treated	Mean erythrocyte volume, μ^3		Plasma prothrombin, per cent		Serum bilirubin, mgm. per cent		Serum cholesterol and cholesterol esters, mgm. per cent		Serum protein, gm. per cent	
		Before	After	Before	After	Before	After	Before	After	Before	After
C.B.	14	..	..	89	100	1.0	0.5	65/113	67/86	7.4	6.2
J.B.	21	89	87	97	100	0.5	0.5	71/137	59/109	5.8	5.9
A.V.	20	90	92	87	82	1.0	0.5	51/108	66/135	6.1	6.5

DISCUSSION

Although heptylaldehyde bisulfite impairs moderately the respiratory activity of human mammary carcinoma *in vitro* (5), and may cause liquefaction necrosis (4, 8) of similar neoplasms in mice, its oral or intravenous administration apparently was without therapeutic effect in the present clinical study. This

be found in the blood of either during the next 3 hours. Likewise, during the course of its administration only traces of bisulfite could be found in the blood of those 3 patients who received the compound by continuous intravenous drip.

Apparently the bisulfite is rapidly hydrolyzed from its heptylaldehyde addition compound *in vivo* and

this is almost immediately oxidized, probably even before the compound reaches the tumor in effective concentration. For these reasons it is desirable to study the effects of other addition products of bisulfite that do not hydrolyze from the carbonyl group so rapidly *in vivo*. These studies now are under way in this Hospital.

CONCLUSION

The daily oral administration of from 1 to 12 gm. of heptylaldehyde bisulfite for from 20 to 231 days to 11 patients with mammary carcinoma that had metastasized to the bones, did not alter signficantly the expected course of the disease. Likewise the continuous intravenous administration of the compound in amounts close to toxic levels failed to influence the course of the disorder in any of 3 instances.

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The Clinical Effects of Aldehyde Bisulfites in Patients with Cancer

II. The Administration of Heptylaldehyde Bisulfite to Patients with Lymphomas

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The respiratory activity of several types of tumor tissue is depressed *in vitro* by the addition of heptylaldehyde bisulfite (3). Because of this observation and because of the therapeutic value of heptylaldehyde bisulfite in mice bearing mammary cancer (4), a clinical study was undertaken in which the compound was administered to women with carcinoma of the breast metastatic to bone (1). In that study, no significantly beneficial effect was noted from either the oral or parenteral administration of the compound. This failure possibly was due to the moderate depression (15 per cent) that heptylaldehyde bisulfite was able to effect *in vitro* on the respiratory activity of breast tissue. On the other hand, the material was found to reduce the respiratory rate of human lymphosarcoma and Hodgkin's tumor by as much as 50 per cent. For this reason alone it appeared necessary to test the effects of the compound in patients with those lymphomatous disorders, and the results of this test form the subject of the present communication.

The methods used in the course of the study are those described in the previous report (1).

Case 1. M. K.—This 27 year white male had widely disseminated reticulum cell lymphosarcoma of 2 years' duration. The disorder became resistant to roentgen irradiation 3 months before the last admission to the hospital. At that time the clinical picture was marked by prominent lymphadenopathy, hepatomegaly and splenomegaly, moderate ascites, and pleural effusion. The sternal marrow and peripheral blood counts were consistent with the diagnosis that was made originally by microscopic examination of a biopsied lymph node.

Heptylaldehyde bisulfite was administered intravenously in increasing amounts until the patient received daily 3.6 gm. (140 cc.). Because this amount repeatedly induced respiratory distress, diarrhea, nausea, and vomiting, the dose was decreased to 3.5 gm. and given daily for the next 17 days. Supportive measures consisted only of occasional thoracenteses, paracenteses, small blood transfusions, high protein diet, and ferric ammonium citrate.

The patient, nevertheless, failed rapidly. Anemia and dyspnea became prominent, and 12 days after the heptylaldehyde bisulfite was discontinued he died from respiratory failure.

Case 2. D. Y.—By microscopic examination of a biopsied lymph node, this 49 year white male was discovered 7 years ago to have lymphosarcoma. Several courses of x-radiation had controlled the disease for only short periods. At the time of the last admission to the hospital the disorder involved principally the palate, stomach, cecum, sigmoid, and rectum. One of only 2 small peripheral lymph nodes was excised for respiratory and microscopic study. The peripheral blood count revealed merely a moderate normochromic, normocytic anemia. The patient had neither splenic nor hepatic enlargement, but the existence of hepatic insufficiency was suggested by a refractory hypoprothrombinemia, hypoproteinemia, and a low-grade hyperbilirubinemia. A routine urine analysis and blood urea nitrogen level revealed no abnormalities.

The patient received heptylaldehyde bisulfite by continuous intravenous infusion. The amount administered through each 24 hour period was gradually increased to a level of 30 gm. (0.5 gm./kg.) daily and continued at that level for 12 days. During this period the patient occasionally complained of nausea, headaches, and small muscular tics.

On the last day of treatment the remaining enlarged peripheral node was removed for microscopic examination and measurement of its respiratory activity; these findings were no different from those observed in the node excised before treatment. Moreover, roentgen examination of the gastrointestinal tract after the administration of heptylaldehyde bisulfite had been discontinued failed to reveal any change. During this study the patient did not develop any further

anemia or evidence of renal or further hepatic dysfunction.

Case 3. A. H.—This 53 year white woman had Hodgkin's disease of from 4 to 6 years' duration. The diagnosis was made originally by biopsy of a lymph node. The sternal marrow was lymphocytic, but the peripheral hemogram consistently revealed only a moderately severe normochromic, normocytic anemia. She had received several courses of x-radiation, but during the past year this therapy had failed to control the disorder.

When admitted to the hospital, the patient was found to have widespread metastases to the bones with massive abdominal and moderate mediastinal lymphadenopathy. Neither the spleen nor the liver was enlarged, and only a few peripheral lymph nodes were palpable. Before treatment was begun one of these nodes was excised for microscopic and respiratory study.

A continuous infusion of heptylaldehyde bisulfite was given for 15 days, during which time the patient received 33 gm. (0.5 gm./kg.) daily. At the end of this period another lymph node was removed. Upon microscopic examination this tissue was found to be free of necrotic changes, and its rate of respiration was of the same order as that of the node previously excised. In the meantime an increase in both the number and size of the metastases to the bones had occurred, and the degree of anemia progressed. Other alterations found at this time were an impaired esterification of cholesterol, a significant degree of hypoproteinemia, and a decreased level of serum calcium. These latter changes may well have occurred spontaneously in the course of the disease, and cannot be attributed to the administration of the heptylaldehyde bisulfite.

TABLE I: THE EFFECT OF HEPTYLALDEHYDE BISULFITE ON PATIENTS WITH NEOPLASTIC LYMPHOMATOUS DISORDERS

Patient	M. K.	D. Y.	A. H.
Diagnosis	Reticulum cell lymphosarcoma	Lymphosarcoma	Hodgkin's disease
Amount of compound given daily	3.5 gm.	30 gm.	33 gm.
Mode of administration	I.V.	Continuous i.v. infusion	Continuous i.v. infusion
Number of days	17	12	15
Effect on clinical course	None	None	None
Effect on microscopic appearance of biopsied neoplastic lymph node	....	"	"
Effect on Q_{O_2} of neoplastic lymph node	....	"	"

TABLE II: THE EFFECT OF HEPTYLALDEHYDE BISULFITE ON PATIENTS WITH NEOPLASTIC LYMPHOMATOUS DISORDERS

Patient	M. K.		D. Y.		A. H.	
Period	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
RBC, million/mm.^{3}	3.20	3.20	3.46	3.53	2.48	1.92
Hemoglobin, gm. per cent	64	65	65	70	52	32
WBC/mm.^{3}	10,200	8,400	6,900	5,700	7,100	4,300
Granulocytes, per cent	84	77	76	60	80	74
Urine	neg.	neg.	neg.	neg.	neg.	neg.
Urea N, mgm. per cent	19.9	25.0	11.7	9.6	10.3	11.4
Bilirubin, mgm. per cent	0.8	0.7	1.8	1.6	1.0	1.0
Serum protein, gm. per cent	5.4	4.9	5.3	5.6	5.7	4.9
Prothrombin, per cent	80	86	98	60	54	57
Mean erythrocyte volume, μ^3	...	...	85	90	88	93
Cholesterol/cholesterol esters, mgm. per cent	83/83	68/115	80/177	80/204	60/133	60.5/68.5

COMMENT

The considerable respiratory depression of lymphosarcoma and Hodgkin's tissue effected by heptylaldehyde bisulfite *in vitro* (3) strongly indicated that the compound should be given a clinical trial. However, the outcome of the present study has failed to indicate any therapeutic result from its administration to patients with lymphomatous disorders. It was known from previous observation that heptylaldehyde bisulfite is rapidly destroyed *in vivo* (3). However, its toxic effect on lymphomatous neoplasms was considered to be so great that if large enough amounts of the compound could be administered some regression of the disease might reasonably be expected. For this reason the heptylaldehyde bisulfite was given in 2 instances by continuous intravenous infusion in an effort to maintain a level just short of that which induced systemic reactions.

In other experiments, to be reported elsewhere, vari-

ous amounts of heptylaldehyde bisulfite fed to rats bearing Murphy lymphosarcoma failed to alter the growth of those tumors or length of life of the animals (2). These observations strongly suggested that no therapeutic success could be expected from the oral administration of the compound to the patients studied. If, as appears to be the case, the *in vitro* toxic effects of heptylaldehyde bisulfite are due to the bisulfite moiety (3), then compounds must be sought that do not dissociate rapidly. Concentrations of these compounds might be maintained at levels that would destroy the neoplastic lymphomatous tissue. Studies of this nature now are under way in this hospital and will form the subject of a subsequent report.

SUMMARY

The intravenous administration of heptylaldehyde bisulfite to patients with neoplastic lymphomatous disorders had no significant effect on their clinical course in the patients studied or on the microscopic appearance or respiratory activity of involved lymph nodes.

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Studies on Tumors of the Testis

I. Water and Electrolyte Content of Testicular Tumors and of Normal, Cryptorchid, and Estrogenized Testis*

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Determination of the water and electrolyte concentrations of tumors of the testis in dogs, and of the normal tissue of origin, was the objective of this study. Testicular tumors had not been studied chemically previous to this work. Since testicular neoplasms are of diverse histological patterns these data were compared with similar analyses of testes from which the germinal epithelium had been eliminated by physiological means, namely, by artificial cryptorchism or by administration of estrogen.

The electrolyte content of tumors has been extensively studied, but many of the older observations are unsatisfactory because of the methods of analysis used, particularly for sodium and potassium. All the articles describe a search for distinct electrolyte patterns that might characterize malignant disease but this paper will demonstrate that no such generalization is feasible in the case of the principal electrolytes, chloride, sodium, and potassium.

The tissue analyses that have been reported by other workers are as follows:

Electrolytes of tumors.—The older data have been presented in a critical review by Shear (25); a complete survey of that literature therefore is not within the scope of this paper.

Water.—The water content has generally been found increased in cancers. In the Jensen rat sarcoma it was stated to vary from 824 gm.[1] in young tumors to 867 gm. in older ones (13), and to average 750 gm. (24). In the Twort mouse carcinoma the water content was 788 to 791 gm. (11), and in a paper on various human and animal cancers values of 660 to 880 gm. (20) were recorded.

Minerals.—Two articles report that the mineral content of tumors is increased over the tissue of origin (22, 23). *Potassium.* Previous communications are in general agreement concerning an increased potassium content of tumors, which was referred to the high cell content. Beebe (2) was the first to report increased potassium values in human tumors, which have been confirmed (2, 5, 8, 9, 20, 22, 23, 26). A high potassium content of Jensen sarcomas was discovered by Clowes and Frisbie (5) and has been found in other tumors of animals (20). The heavy isotope of potassium, K^{41}, is decreased in the Jensen sarcoma (14) and in human cancers (15), in comparison with the mineral potassium as contained in ordinary potassium chloride. *Sodium.* From the standpoint of methodology the data on sodium content are less reliable than those for potassium. Increased values in tumors have been found with older analytical methods (2, 20, 22), and from study of the emission spectrum of sodium (3). *Lipids.* The previous studies of tissue electrolytes in cancer have not been corrected for fat, a procedure that Hastings and Eichelberger (10) found to be essential in order to eliminate large fluctuations in the analytical data on muscle. In transplants of Jensen sarcoma to rats (13) total cholesterol increases with the age of the transplant, while the content of neutral fat decreases.

TABLE I: ANALYTICAL DATA IN THE LITERATURE ON THE WATER AND ELECTROLYTE CONTENT OF TESTIS

Values expressed per kilo of tissue.

Species	Water, gm.	Fat, gm.	Chloride, mM	References
Man	866.1	45.1	63.7	(16)
Rat	867.0	9.8–11.0	63.66	(19)
Rabbit	857.0	10–11	52.9–58.7	(19)
Rat	..	..	62.6	(4)
Dog	..	..	52.7	(4)
Dog	..	..	58.3–62	(6)
Man	..	..	76	(4)
Cattle	860.0	15.55	..	(21)
Cat	..	..	60.0	(1)

Water and electrolytes of testis.—The available data are summarized in Table I. The adult testis is high in water. Rabbit testis contains sodium, 47.5 millimols per kilo (19). In his classical study on the organs of a suicide Magnus-Levy (16) found nitrogen values of 1.37 gm. per hundred grams. Spontaneous tumors of the testes occur frequently in dogs. We find it useful

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[1] Data in grams per kilogram of tissue.

to classify them broadly in two main types. *Type I,* interstitial cell tumors, in the gross are orange-yellow in color, encapsulated, usually small, and have a tendency to undergo necrosis with liquefaction; microscopically they are seen to consist of vacuolated cells containing much material that stains with Sudan dyes. *Type II,* lobulated tumors, contrast sharply with interstitial cell tumors; on section they are white and nodular, often large, and do not undergo necrosis; microscopically various cell types are found, some of which resemble cells of the normal germinal epithelium. Because the histogenesis of testicular tumors is not clear, we designate as lobulated tumors all neoplasms of the testis that are not of the interstitial cell type. This includes tumors resembling human seminomas, those resembling normal Sertoli cells (tubular adenomas), and also other tumors of less differentiated cell type.

Table II: Water and Electrolyte Content of Normal Adult Canine Testes

The values are given per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	Ca, mM	Mg, mM	N, gm.
Mean	873	20.3	60.3	45.6	90.9	0.85	5.0	16.6
Standard deviation	4.4	3.18	3.0	5.2	9.6	..	..	1.6
Highest value	880.1	23.9	70.4	56.0	105.4	0.9	5.7	20.5
Lowest value	862.0	14.7	55.4	38.2	73.5	0.8	4.5	14.2
No. of determinations	26	26	26	17	18	3	3	16

METHODS

The following tissues were available for analysis: 26 normal testes; 8 interstitial cell tumors; 14 lobulated tumors from 7 dogs; the testes of 4 dogs in which artificial cryptorchism was produced and of 2 dogs injected with estrogen. The animals were killed by an electric current and the tissues obtained immediately for analysis. To eliminate connective tissue as much as possible, the tunica albuginea was incised and the tubular mass of the testis separated from it by blunt dissection and deposited in a weighing bottle. Samples of all of the tissues analyzed were verified histologically.

In the cryptorchism experiments the left testis of 4 normal dogs was excised surgically and the right testis inserted and secured in the peritoneal cavity by suture of the inguinal canal. The left testis was removed surgically from 2 dogs, which were then injected with diethylstilbestrol, 3 mgm. twice each week. Both experiments were terminated after 36 days, and the remaining testis was recovered for chemical analysis.

The chemical methods were those employed in a comparable study of the kidney by Eichelberger and Bibler (7); nitrogen was determined by Kjeldahl's method.

RESULTS

Normal testis.—The results are given in Table II. Features of interest are the high water content and the low percentage of neutral fat. While the chloride concentration of 21 testes in the group of 26 varied between 57 and 63 mM per kilo, 2 of the testes had chloride concentrations of 70 mM per kilo. The average chloride content of 60.3 mM is greatly in excess of the sodium value of 45.6 mM per kilo of fat-free testes, which is exceptional for most tissues of the body. It will be noted that in testis, as in other tissues, potassium is the predominating univalent base and magnesium the bivalent base.

A similarity of analytical results occurred between right and left testis; for example, chloride values differed at most by 2.4 mM per kilo. Paired normal organs in the body usually have essentially the same chemical characteristics; for example, the values for the right and left kidney from the same animal have been found to be the same (7).

The testes of newly caged dogs frequently undergo dissolution leading to temporary atrophy, which is reversible (12). In 7 instances testes with severe dissolution were encountered. There were no significant changes in the fat or chloride content, but in each instance of atrophy decreased amounts of water (mean 861 gm.) and of potassium (average 75 mM) and increased amounts of sodium (average 64 mM) were found.

Table III: Water and Electrolyte Content of Testes from which Tumors were Removed

The values are given per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM
Mean	868.8	19.0	67.2	57.4	85.2
Highest value	886.4	29.0	82.2	66.7	108.5
Lowest value	850.9	11.4	49.1	49.1	70.6
No. of determinations	18	17	18	7	7

Testes from which tumors were removed.—High chloride values (66 to 82 mM per kilo) were found in the remaining normal testis of 10 dogs in a group of 18 from which tumors had been excised at autopsy (Table III). In 8 instances the chloride values were within normal limits.

Interstitial cell tumors.—Obviously liquefied areas were discarded, but the removal was sometimes in-

complete. The analytical data are given in Table IV. The high content of neutral fat is noteworthy. Chloride and sodium concentrations were increased, potassium and water were decreased. Histologically these tumors consist of vacuolated cells resembling typical Leydig cells of the normal testis.

TABLE IV: WATER AND ELECTROLYTE CONTENT OF INTERSTITIAL CELL TUMORS

The values are given per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM
Average	846	87.1	66.8	58.1	72.2
Standard deviation	14.6	34.4	10.6	..	..
Highest value	865.7	148.9	90.8	58.6	87.5
Lowest value	820.2	39.7	57.7	57.6	56.9
No. of determinations	8	8	7	2	2

Lobulated Tumors, Type I.—These white solid tumors were always free from necrosis and liquefaction. They were classified further into two groups, according to whether their fat content was lower or higher than normal. *Type I.* Those with low fat content, mean value 5.2 ± 2.3 gm. per kilo (Table V) consisted of sheets of poorly differentiated cells with minute droplets of sudanophilic material between them; the cell type of one of these tumors resembled normal spermatagonia. The prostate glands of all these dogs contained normal tall cylindical epithelium.

Lobulated tumors, Type II.—The solid tumors with high fat content, mean value of 56.8 ± 18.9 gm. (Table VI) were obtained from 2 dogs. In Dog D, the cells contained large fat droplets in the nuclei; the prostatic epithelium was replaced by squamous cells with much intra-alveolar desquamation. These prostatic changes are evidence of the production of abnormally large amounts of estrogen. The tumor in Dog C, a tubular adenoma of the testis, was unaccompanied by estrogenic stimulation of the prostate.

TABLE V: WATER AND ELECTROLYTE CONTENT OF LOBULATED TUMORS OF LOW FAT CONTENT

The values are given per kilo of fat-free tissue.

Dog	Tumors	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	Ca, mM	Mg, mM	N, gm.
IB-1	Undifferentiated carcinoma	846.1	7.0	54.4	34.9	123.5			
IB-2	"	846.9	19.1	58.8	60.9	77.6			
828-3	"	837.0	4.4	54.2	29.5	103.4			
828.4	"	833.1	2.3	50.2	25.1	103.1	0.7	6.7	
828.5	"	837.7	2.3	48.9					21.0
625-6	Seminoma	840.0	4.0	49.6	34.0	115.6			
601-7	"	838.0	2.0	53.0					
703-8	"	835.0	2.4	52.8					23.2
703-9	"	836.0	3.1	54.8					22.0
Mean		838.9	5.2	53.0	36.9	104.6			22.0
Standard deviation		4.4	2.3	2.9	12.5	15.8			

TABLE VI: WATER AND ELECTROLYTE CONTENT OF LOBULATED TUMORS WITH HIGH FAT CONTENT

The values are given per kilo of fat-free tissue.

Dog	Tumors	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	N, gm.
C-1	Tubular adenoma	855.6	68.0	54.5	59.7	53.8	20.8
C-2	" "	836.5	46.9	65.6	63.1	80.9	22.5
C-3	" "	844.0	20.7	55.7	56.6	75.1	21.6
D-4	Undifferentiated carcinoma with estrogenic effects	828.6	72.9	59.7			
D-5	" "	836.4	50.4	55.3	33.0	129.5	21.8
D-6	" "	748.2	75.1			94.7	20.8
Mean		829.9	56.8	58.2	50.8	86.8	21.7
Standard deviation		24.6	18.9	3.8			0.2

In both types of lobulated tumors the water was abnormally low and the nitrogen values were higher than in normal testes. In the group with high fat content chloride concentration was similar to normal testis, sodium was slightly increased, and potassium slightly decreased. In the group with low fat content sodium and chloride were significantly decreased, and potassium increased, relative to normal testis.

Cryptorchism and estrogen.—In both cases the germinal epithelium was completely abolished and the tubules became atrophic, being lined with a single layer of cells. The relative fat content was unchanged, but in all instances except one there was a decrease of

water (Table VII). In the cryptorchid group there was no significant change in chloride, while estrogen was followed by an increase of chloride. In each instance potassium was decreased and sodium increased by atrophy.

DISCUSSION

Normal testis is a complex tissue, comprised of many different types of cells that vary in structure and function. Since the two main types are the interstitial cells, which are secretory, and the epithelial cells of the tubules, which are largely sex cells, it is not surprising that the testis should differ from other tissues of the body in water content and electrolyte concentration.

If a quantitative interpretation of the analytical data for testis expressed as exact volumes of extracellular and intracellular phases were possible, more useful conclusions could be drawn. That all the sodium and chloride are not extracellular has been demonstrated by numerous investigators. In 1938 Amberson and his group (1) showed that when plasma chlorides had been greatly reduced in concentration, and all chlorides were reduced in consequence, testis retained 18 per cent of its chloride. In 1941 Manery and her associates (17, 18), working with radioactive isotopes, demonstrated that injected radioactive sodium entered only two-thirds of the total sodium space and radioactive chloride only one-half of the chloride space, indicating intracellular chloride and sodium. Only tentative conjectures, therefore, can be made at this time. Since the potassium values of 90.9 mM per kilo of testis are not different from those found in the more simple, skeletal muscle (97.1 mM), and if this ion is indicative of the size of the intracellular phase, this phase in testis must be approximately the same as that found in muscle.

Since sodium and chloride are confined to the extracellular phase in skeletal muscle, and exist there as an ultrafiltrate of serum, the concentration of these ions should indicate the size of the extracellular phase in testis. On the other hand, if all the sodium and chloride of testis were extracellular, and existed as an ultrafiltrate of the serum, the ratio of sodium to chloride should approximate the serum value of 1.26. Instead a value of 0.73 was found, indicating that all

TABLE VII: WATER AND ELECTROLYTE CONTENT OF CRYPTORCHID AND ESTROGENIC TESTES COMPARED WITH THE NORMAL. DURATION OF EXPERIMENTS: 36 DAYS

Values expressed per kilo of tissue.

Dog	Testis	State	Weight, gm.	Water, gm.	Fat, gm.	Chloride, mM	Sodium, mM	Potassium, mM
			(A) CRYPTORCHISM					
750	Left	Normal	12	874.6	18.1	58.6	..	93.4
	Right	Cryptorchid	6	856.1	14.7	61.6	60.3	76.8
608	Left	Normal	8	866.4	21.6	63.3	46.3	86.2
	Right	Cryptorchid	5	842.5	25.6	60.0	58.3	74.8
664	Left	Normal	14	876.9	19.2	60.8	39.0	95.0
	Right	Cryptorchid	6	852.9	16.2	58.4	57.8	77.6
696	Left	Normal	18	880.1	22.9	70.4	56.0	84.5
	Right	Cryptorchid	10	869.0	28.2	71.2	63.9	75.5
			(B) ESTROGEN					
827	Left	Normal	13	867.8	20.1	55.4	39.4	100.5
	Right	Estrogen	6	842.2	21.6	70.1	..	84.1
825	Left	Normal	16	872.3	18.2	56.7	47.5	95.4
	Right	Estrogen	10	880.7	25.1	65.4	60.7	72.3

TABLE VIII: SUMMARY OF WATER AND ELECTROLYTE CONTENT OF TESTIS

The figures are mean values per kilo of fat-free tissue.

	Water, gm.	Fat, gm.	Cl, mM	Na, mM	K, mM	Ca, mM	Mg, mM	N, gm.
Normal testis	873.0	20.3	60.3	45.6	90.9	0.86	5.0	16.6
Testis from which tumors were removed	868.8	19.0	67.2	57.4	85.2			
Interstitial cell tumors	846.0	87.1	66.8	58.1	72.2			
Lobulated tumors, Type 1 (low fat)	838.9	5.2	53.0	36.9	104.6			22.0
Lobulated tumors, Type 2 (high fat)	829.9	56.8	58.2	50.8	86.8			21.3
Cryptorchid	852.9	16.2	62.8	60.1	76.1			
Estrogen	842.2	21.6	67.8	60.7	83.2			

the sodium and chloride of testis is not extracellular and that there are cells in which the intracellular chloride exceeds the intracellular sodium.

When comparisons of findings from normal testis were made with those obtained on testis from which the germinal epithelium had been eliminated by cryptorchism or estrogen, uniform differences were found. In all, the total mass of the testis decreased approximately 50 per cent, and the water content decreased also. At the same time, in the testis of cryptorchism the chloride concentration did not change significantly. The sodium and potassium values should be considered in their relationship to each other. The sodium values were definitely increased beyond the limit of experimental error, and since a decrease in total water and little change in chloride indicate that there has been no increase in the extracellular fluid there is strong indication that sodium ions in excess of the normal amount have entered the cells. The potassium loss was even greater than the sodium gain. These findings indicate that the sum of the sodium and potassium values of these testes is approximately the same as in the normal testis, but elimination of the germinal epithelium seemed to cause the exit of potassium ions from the cells and the entrance of sodium ions.

In contrast to previous findings in neoplasia, the testicular tumors without exception contained less water than did normal testis. Concomitant with this increase of total solids the nitrogen content of tumors, mean value 21 to 22 gm. per kilo, was increased over the corresponding values of normal testis, 16.6 gm.

The sum of the electrolytes in normal testis varied between 123 and 146 mM. per kilo, while in the neoplasm these values ranged from 115 to 158.4 mM. Always when the sodium was increased in amount, potassium values were lowered. In interstitial cell tumors necrosis with liquefaction often occurred, and these growths were associated with increased sodium and decreased potassium. This finding evidently is the result of an increased amount of fluid. In a single instance, not included in the table, massive liquefaction of an interstitial cell tumor was found. Analysis of this entire tumor revealed the following values per kilo; chloride, 90.8 mM.; sodium, 130 mM.; and potassium, 9.46 mM. In the lobulated tumors with high fat content, apparently as cellular as other lobulated tumors, sodium was generally increased and potassium reduced.

SUMMARY

1. The normal testis of dogs has a high water and low neutral fat content. For normal testis the mean values per kilo of fat tissue were as follows: total water, 873 ± 4.4 gm.; chloride, 60.3 ± 3.0 mM; sodium, 45.6 ± 5.2 mM; potassium, 90.9 ± 9.6 mM; calcium, 0.86 mM; magnesium, 5 mM; and nitrogen 16.6 ± 1.6 gm. The values for right and left testis from the same animal were similar.

2. In testes with atrophy, whether from caging, cryptorchism, or estrogen, water and potassium values were decreased and sodium was increased in amount. Chloride values were at a normal level in the atrophy from caging and cryptorchism but were slightly increased after estrogen. These results indicate that the sum of the sodium and potassium values is the same as found in normal testis, but the elimination of the germinal epithelium seemed to cause the exit of potassium ions from the cells and an entrance of the sodium ions.

3. The neutral fat content of interstitial cell tumors is greatly increased; mean value 87.1 ± 34.4 gm. per kilo was obtained.

4. The lobulated white tumors of the testis were of two types with respect to the neutral fat content. A type with low fat content, mean value 5.17 ± 2.3 gm. per kilo, consisted of sheets of poorly differentiated cells (pathological classification, undifferentiated carcinoma and seminoma). In the testes of 2 dogs, 6 large tumors were found with large amounts of fat (mean value 56.8 ± 18.9 gm. per kilo); in one of these dogs whose tumors were undifferentiated carcinomas there was estrogenic stimulation of the prostate not present in the other dog, whose tumors were tubular adenomas.

5. In dogs, with respect to the normal testis, testicular carcinoma uninvolved by necrosis was characterized by decreased water and increased solids and nitrogen values. The sodium and potassium content was irregular and the interpretation of the findings is complex. When necrosis and liquefaction were present, sodium was increased and potassium decreased (interstitial cell tumors). Lobulated testicular tumors with low fat content had increased potassium and decreased sodium content, while in the group of lobulated tumors with high fat content, equally cellular and free from necrosis, this relationship was reversed.

CONCLUSION

Typical patterns of the content of water, fat, and electrolytes are described for normal testis and various physiological types of atrophy of the germinal epithelium. While definite and categorical changes occur in neutral fat, water, solids, and nitrogen, quantitative generalizations concerning electrolytes cannot be made for the testicular cancer of dogs because of the wide deviations.

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Abstracts

Experimental Research, Animal Tumors

Transplantable Osteogenic Sarcomas Induced in Rats by Feeding Radium. DUNLAP, C. E., AUB, J. C., EVANS, R. D., and HARRIS, R. S. [Harvard Med. Sch., Harvard Cancer Commission, Boston, Mass., and Massachusetts Inst. of Technology, Cambridge, Mass.] *Am. J. Path.*, **20**:1-21. 1944.

Thirteen male Wistar rats were fed 100 mgm. of radium. Within 10 days the animals had excreted 95% of the radium, and the average amount retained at the end of 10 months was 2 mgm. Nine of the animals manifested osteogenic sarcomas in the vertebrae or pelvic bones after 253 to 426 days. Two of the 9 primary tumors metastasized, and 3 of the growths were successfully transplanted. Both primary and transplanted growths contained large amounts of alkaline phosphatase as shown by chemical and microscopical methods.

In addition to the neoplasms, widespread necrosis of bone was present in all of the rats, and atypical new bone formation was commonly found, as well as hypoplasia of the bone marrow and extensive hemosiderosis.

The authors suggest that the partially frustrated attempts to regenerate bone in regions of radium necrosis may be responsible for the ultimate appearance of the osteogenic sarcomas. They conclude that the sarcomas and the other pathological changes produced experimentally in rats reproduce those seen in human beings as a consequence of radium poisoning. Six figures are included.—J. G. K.

Further Report on Malignancy Observed in Rats Injected with Crude Ether-Extracted Wheat Germ Oil. ROWNTREE, L. G., and ZIEGLER, W. M. [Philadelphia Inst. for Med. Research, Philadelphia General Hosp., Philadelphia, Pa.] *Proc. Soc. Exper. Biol. & Med.*, **54**:121-123. 1943.

The paper is concerned with an attempt to confirm and extend original experiments of the same author in which crude, ether-extracted, wheat germ oil, either fed to rats or injected intraperitoneally, had been followed by the appearance of tumors in the abdominal cavity. So far, efforts by 6 different laboratories to duplicate and confirm the results have been completely unsuccessful. The authors now report that they have been unable to reproduce the results of the feeding experiments. On the other hand new tests in which 4 different preparations of wheat germ oil were injected (30 cc. in 30 injections over a period of about 56 weeks) intraperitoneally into small groups of rats of various breeds have been positive. In a total of 41 rats, 11 developed tumors, presumably in the abdominal cavity, 220 to 670 days after the beginning of the injections. Of these tumors 8 were sarcoma, 3 were carcinoma.

In control experiments the injection of pressed wheat germ oil into 5 rats, of sesame oil into 3 rats, and of sesame oil plus 2% starch into 3 rats, gave negative results. One sarcoma developed in a group of 3 rats injected with sesame oil containing 2% starch and 2% sterols.

The animals that received the test oils suffered from peritonitis, their abdominal organs becoming embedded in a single mass of fibrous adhesions. One-third of the animals developed an abdominal fistula, with purulent discharge in some cases. These lesions were not present in the control animals.—A. C.

Studies on Hepatomas. I. Size and Spacing of Multiple Doses in the Induction of Carbon Tetrachloride Hepatomas. ESCHENBRENNER, A. B. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:385-388. 1944.

Thirty doses of carbon tetrachloride in olive oil were administered to mice by stomach tube. A variation in the numerical incidence of hepatomas at a given time was observed to be related both to the total amount administered and to the interval elapsing between successive doses. The influence of each of these two dependent variables is approximately of the same order so that the effects of variations of one of these must never be considered alone but always in terms of the other. The numerical incidence of hepatomas at a given time was found also to be related to the tumor-development time.—Author's summary.

A Possible Mode of Action of Benzpyrene as a Typical Chemical Carcinogen. WEIGERT, F. [The Mount Vernon Hosp., Northwood, Middlesex, England] *Trans. Faraday Soc.*, **39**:418-419. 1943.

Fluorescence- and absorption-spectrography and fluorescence-chromatography suggest that benzpyrene before being metabolized in the animal body to 5,8-benzpyrene-quinone and 8-hydroxy-benzpyrene passes through two intermediate stages.

The author sees an analogy between the existence of these chemically intermediate stages and the intermediate stages in carcinogenesis evoked in tissues by benzpyrene. (See abst. in *Cancer Research*, **4**:199. 1944.)—I. H.

The Effect of Aromatic Compounds upon the Ascorbic Acid Content of the Liver in Mice. KENNAWAY, E. L., KENNAWAY, N. M., and WARREN, F. L. [Royal Cancer Hosp. (Free), London, England] *Cancer Research*, **4**: 367-376. 1944.

3,4-Benzpyrene injected in sesame or arachis oil subcutaneously or intraperitoneally, and other carcinogenic compounds (9,10-dimethyl-1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 1,2,5,6-dibenzphenanthrene, cholanthrene, methylcholanthrene) injected in arachis oil intraperitoneally, caused an increase in the concentration of ascorbic acid in the liver of mice. Dimethylaminoazobenzene produced a less definite increase, and the 3 noncarcinogenic compounds tested (naphthalene, anthracene, phenanthrene) caused no increase at all. 1,2-Benzanthracene had an effect similar to that of the strongly carcinogenic compounds named above, while 9,10-dimethylanthracene, which is carcinogenic to the skin, gave a negative result. The identity with ascorbic acid of the reducing substance esti-

mated in these experiments was shown by the use of ascorbic acid oxidase from vegetable marrow.

The glutathione content of the liver was not affected distinctly by the compounds (3,4-benzpyrene, dimethylaminoazobenzene, anthracene) tested in this respect.

In male mice injection of sesame oil subcutaneously or intraperitoneally, and of arachis oil intraperitoneally, caused an increase in the weight of the liver, and the addition of carcinogenic hydrocarbons to the oil caused a further increase.

Some evidence was obtained that these increases in ascorbic acid in the liver occur only if there is some deficiency in the store of the compound already present.—Authors' abstract.

The Role of Calcium in Carcinogenesis. Carruthers, C., and Suntzeff, V. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Med. Sch., St. Louis, Mo.] *Science,* **99**:245-247. 1944.

It was found that normal and benzene-treated mouse epidermis contained 0.042 to 0.044 mgm. of calcium per 100 mgm. of tissue. Within 10 days following an application of methylcholanthrene to the skin, the calcium content fell to 0.019 mgm. and remained at about this level for 60 days. In non-necrotic material of a transplantable carcinoma derived from methylcholanthrene-treated epidermis the calcium content was still lower, being 0.009 mgm. per 100 mgm. of tissue. It is concluded that reduction of calcium is an important feature of experimental carcinogenesis; it occurs immediately after application of the tumor incitant and again after the cells have become malignant.—R. B.

Carcinogenic Hydrocarbons and Synthetic Oestrogens. Martin, R. H. [Dyson Perrins Laboratory, Oxford, England] Chem. and Indust., No. 10:94-95. 1944.

A resemblance between the carbon skeletons of (I) the estrogenic compound β,δ-di-(*p*-hydroxyphenyl)-γ-ethylhexane (Blanchard, Stuart, and Tallman, *Endocrinology,* **32**: 307. 1943), and of the carcinogenic compounds 9,10-dimethyl-1,2-benzanthracene and cholanthrene, is pointed out. The hydrocarbon corresponding to (I) would be of interest. There is a similar relationship between 20-methylcholanthrene and the as yet unknown 4′,9,10-trimethyl-1,2-benzanthracene.—E. L. K.

Studies in Cancer. VIII. Stilbestrol and Certain Steroids in Relation to Tumor Growth Resistance. Howard, J. W., Janzen, L. J., and Salter, W. T. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **4**:337-344. 1944.

In appropriate strains of mice, resistance against sarcoma can be enhanced by massive doses of estrogens, *i.e.,* estrone, progesterone, diethylstilbestrol, or stilbestrol.

The tentative indications of these studies are twofold: First, it may be that animals with a high incidence of spontaneous tumor cannot have their immunity mechanism enhanced by an estrogen. Because several investigators have suggested that cancer is the result of a perverted sterol metabolism, such a finding would be highly significant. Second, in regard to the effect of the estrogen, stilbestrol, the indication is that if it is effective in enhancing immunity against neoplasm, this result occurs only by virtue of its transformation through female enzyme systems; in other words, it acts only because it is the precursor of an estrogen. This hypothesis requires more general information concerning the metabolism of stilbestrol and its mode of action in the female.—Authors' abstract.

On the Role of Thymus, Spleen, and Gonads in the Development of Leukemia in a High Leukemia Stock of Mice. McEndy, D. P., Boon, M. C., and Furth, J. [Cornell Univ. Med. Coll., New York, N. Y.] *Cancer Research,* **4**:377-383. 1944.

Removal of the thymus from mice of a high leukemia stock (AK) at 31 to 71 days of age resulted in a reduction of the incidence of spontaneous leukemia from 77% to 8% in females, and from 61% to 11% in males.

Leukemia is more common in female than in male mice. The incidence of this disease was lowered from 74% to 45% by ovariectomy at 23 to 56 days. Among males subjected to orchidectomy at 20 to 56 days the incidence of leukemia was 60% as compared with 52% among the controls of this experimental series.

Splenectomy at 28 to 48 days did not significantly alter the incidence of spontaneous leukemia.

The role of thymus, spleen, and gonad in the causation or evolution of spontaneous leukemia is discussed in the light of the data here presented.—Authors' summary.

The Effect of Adrenalectomy on the Susceptibility of Rats to a Transplantable Leukemia. Sturm, E. and Murphy, J. B. [Rockefeller Inst. for Med. Research, New York, N. Y.] *Cancer Research,* **4**:384-388. 1944.

In the experiments reported removal of the adrenals reduced the natural resistance of old rats and the induced resistance of young rats to a transplantable lymphatic leukemia. Inoculation of intact, middle-aged animals of a special strain resulted in 43.5% mortality, while 89.7% of adrenalectomized rats of the same strain and age developed the disease. Young rats with induced resistance gave 33.9% takes following inoculation. Animals in which the adrenals were removed subsequent to the resistance-inducing treatment were more than 90% susceptible, while in another group, adrenalectomized before the immunizing treatment, 78.8% died of leukemia. A different strain of rats, highly resistant to the transplanted leukemia used in the tests, became 100% susceptible following removal of the adrenals.

A prominent feature in the adrenalectomized rats is the regeneration of the retrogressed thymus in old animals and an active stimulation of this gland in young ones. It is suggested that the greater receptivity of adrenalectomized rats to transplanted leukemia is the result of the action of the same stimulating factors on the malignant lymphoid cells.—Authors' summary.

The Effect of Adrenal Cortical and Pituitary Adrenotropic Hormones on Transplanted Leukemia in Rats. Murphy, J. B., and Sturm, E. [Rockefeller Inst. for Med. Research, New York, N. Y.] *Science,* **99**:303. 1944.

Injections of adrenal cortex hormones or of pituitary adrenotropic hormone brought about a definite increase in the survival of rats inoculated with a transplantable lymphatic leukemia. In various experiments 20% to 75% of the hormone-treated rats survived after inoculation with leukemic cells compared with a survival of 0% to 10% among control rats.—R. B.

Atypical Cell Proliferation in the Anterior Lobe Adenomas of Estradiol-Treated Rats. SELYE, H. [McGill Univ., Montreal, Canada] *Cancer Research,* **4**:349-351. 1944.

The anterior lobe adenomas elicited in rats by long-continued estradiol treatment may exhibit signs of atypical cell proliferation. In the present study such adenomas were found to contain polynuclear giant cells and an unusually large number of mitotic figures. The cytoplasm of the giant cells was basophilic and frequently contained pigmented granules or crystalloid inclusions. Metastases or other signs of malignancy were not observed.—Author's summary.

Extensive Breeding as an Adjunct to Mammary Gland Carcinoma Susceptibility in Mice. STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] *Proc. Soc. Exper. Biol. & Med.,* **53**:257-258 1943.

Reciprocal crosses were made between mice of the JK strain (cancer resistant) and of the C3H strain (cancer susceptible). The F_1 progeny were mated in various ways and used as breeders for their entire life span, the sexes being kept together. Maximal nutritional conditions were maintained. Seventeen of the 24 F_1 females from mothers of the JK strain, eventually developed spontaneous carcinoma of the mammary gland, at an average age of 476.2 days. In the reverse cross, 25 of the 30 F_1 females from C3H mothers developed similar tumors at an average age of 424.6 days. The difference in susceptibility between the two F_1 female groups is probably not significant mathematically. Thus, in this type of cross, between the C3H and JK strains, extensive breeding seemed to be an effective way of bringing about the development of spontaneous tumors of mammary tissue, and the role of the "milk influence" was not clear. Many influences appear to affect the occurrence of mammary tumor, among them genetic, hormonal, chemical, dietary factors; normal and abnormal activities such as forced breeding and extensive breeding; and possibly somatic mutation. The role of these influences may differ under different experimental conditions.—M. B.

Importance of Genetic Influence on the Occurrence of Mammary Tumors in Virgin Female Mice. HESTON, W. E., and ANDERVONT, H. B. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:403-407. 1944.

Two inbred strains of mice, A and C3H, were studied. Both are high-mammary-tumor strains but differ in that whereas both the breeding and virgin females of strain C3H have a high incidence of mammary tumors, only the breeding females of strain A have a high incidence. Few mammary tumors develop in the virgin strain A females.

Reciprocal crosses between the 2 strains demonstrated that this difference was due largely to a difference in genic complex influencing tumor development. In degree of susceptibility to mammary tumors, the F_1 hybrid virgin females were intermediate between the parent strains although more like the parent strain C3H.

Reciprocal foster nursing between the 2 strains did not give conclusive evidence of a difference in the mammary-tumor agent in the milk of the 2 strains.—Authors' summary.

The Heterologous Transplantation of Human Cancers. GREENE, H. S. N., and LUND, P. K. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research,* **4**:352-363. 1944.

A series of 10 human cancers including a fibrosarcoma of the chest wall, an adenocarcinoma of the salivary gland tissue, a chondromyxosarcoma of the larynx, a malignant melanoma, an epidermoid carcinoma of buccal mucosa, an adenoacanthoma of the urethra, a mammary fibrosarcoma, an undifferentiated carcinoma of the lung, an epidermoid carcinoma of the lung, and a chordoma have been successfully transferred to the anterior chambers of the eyes of guinea pigs. The transplants grow progressively in the alien host and bear a close histological resemblance to the original tumors.—Authors' summary.

Action of Bacterial Toxins on Tumors. V. Immunological Protection Against Tumor Hemorrhage. ZAHL, P. A., HUTNER, S. H., and COOPER, F. S. [Haskins Labs., New York, N. Y.] *Proc. Soc. Exper. Biol. & Med.,* **54**:48-50. 1943.

White Rockland mice were immunized with successive doses of a *Shigella paradysenteriae* endotoxin preparation. Ten to 15 days after the first injection, sarcoma 180 was implanted subcutaneously. Animals bearing 7 day old tumors, together with a control series of nonimmunized tumor-bearing mice were given intraperitoneal doses of the preparation used for immunization; 6 hours later the tumors were examined for hemorrhage.

In the control animals, hemorrhage was induced at all doses greater than 1/200 of an LD50. In the immunized animals, all doses smaller than one LD50 failed to produce hemorrhage.—M. B.

Immunity Reactions Obtained with a Transmissible Fowl Tumor (Olson). BURMESTER, B. R., and PRICKETT, C. O. [Agricultural Research Admin., U. S. Dept. of Agriculture, East Lansing, Mich.] *Cancer Research,* **4**:363-366 1944.

A high proportion of local tumors was produced in 312 white leghorn chickens when a transmissible fowl tumor (Olson) was implanted intramuscularly, subcutaneously, intradermally, or by dermic scarification. All birds surviving inoculation showed a regression of the tumor and immunity to subsequent implantation of the same tumor strain. The immunity obtained is of particular interest because it appears in all birds that have survived active growth of the neoplasm, it cannot be overwhelmed by very large or repeated doses of the same agent, and it is present over a prolonged experimental period.—Authors' abstract.

Survival of Normal Cells in Penicillin Solutions Lethal to Malignant Cells. CORNMAN, I. [Wistar Inst., Philadelphia, Pa.] *Science,* **99**:247. 1944.

Rat and mouse sarcoma cells in tissue culture were severely damaged or killed by penicillin in one-third to one-half the concentration necessary to produce an equivalent injury in normal fibroblasts. Transplants of severely damaged rat sarcoma cultures into rats failed in most cases to produce tumors.—R. B.

A Transplantable Osteogenic Sarcoma Originating in a C3H Mouse. BARRETT, M. K., DALTON, A. J., EDWARDS, J. E., and GREENSTEIN, J. P. [National Cancer Institute, Bethesda, Md.] *J. Nat. Cancer Inst.,* **4**:389-402. 1944.

The history, pathology, cytology, and phosphatase activity of a spontaneous, transplantable, osteogenic sarcoma

are reported. The primary tumor, the early generations of subcutaneous transplants, and the pulmonary metastases arising from them consisted of malignant osteoblasts, osteoid tissue, and true bone. The early generations of transplants were characterized by a moderate growth rate and the possession of a high alkaline phosphatase activity. With a rapid increase in growth rate in later generations, the high alkaline phosphatase activity and the capacity of forming osteoid tissue characteristic of the early generations were either inhibited or lost. Coincident with the increase in growth rate, early metastases to regional lymph nodes as well as to the lungs were noted. The significance of these observations as they relate to the question of the cell of origin in osteogenic sarcoma is discussed.—Authors' summary.

Adamantoblastomas in the Slye Stock of Mice. Zegarelli, E. V. [Columbia Univ., New York, N. Y.] *Am. J. Path.*, **20**:23-87. 1944.

Seventy-nine mice provided 103 adamantoblastomas for study. The growths were cystic or solid or both. They originated in the embryonal cells comprising the outer epithelial layer of the enamel organ. Infiltration of surrounding tissues (bone, muscle, lymphatics) was frequently observed in the types of adamantoblastoma that contained solid epithelial masses, and metastases to the submaxillary lymph nodes were found in 2 cases. Forty-nine figures illustrate the character and origin of the growth and stages in its development.—J. G. K.

The Nearer Causes of Cancer. Rous, P. [Rockefeller Inst. for Med. Research, New York, N. Y.] *J. A. M. A.*, **122**: 573-581. 1943.

The action of the many carcinogenic agents, mechanical, chemical, estrogenic, and so forth, is to cause a chronic cellular disturbance on the basis of which neoplastic change may or may not supervene after a considerable lapse of time, the outcome depending on the potentialities of the tissues acted upon. The only carcinogenic agents that are known to produce tumors by direct action are the neoplastic viruses. These directly change the cells they infect into neoplastic cells without giving rise to any intermediate tissue disturbance, and they evidence great cellular specificity in their action. The difficulties of supposing that viruses cause the generality of tumors has forced several assumptions. One of these is that the body itself may carry indigenous viruses. Now and again these may become so changed in response to intercurrent conditions as to work on the cells with which they are associated with the result that they become tumor cells. Such viruses may reach young creatures in early life, either in utero or during suckling, resulting in an inapparent infection. If a provocative carcinogen happens to work on cells with which such a virus is associated, it may undergo variation and give rise to a tumor. Recent discoveries with the "milk influence" have provided an instance that embodies this concept.—M. E. H.

Clinical and Pathological Studies

Radiation—Diagnosis and Therapy

A Study of Roentgen-Ray Distribution at 60-140 Kv.P. Atlee, Z. J., and Trout, E. D. [Chicago, Ill.] *Radiology*, **40**:375-386. 1943.

Studies of the distribution of radiation about x-ray tubes at various kv.p. and with various factors were made. It was concluded that tubes used for therapy should not be used for radiography because of changes in intensity pattern due to roughening of the target. The target angle of a superficial therapy tube should be at least 30°. A curved or convex target face effects an improvement in field distribution.—R. E. S.

Radiation Therapy in Carcinoma of the Breast. Bouchard, J. [Royal Victoria Hosp., Montreal, Canada] *Canad. M. A. J.*, **49**:382-387. 1943.

The paper includes a general discussion of the various modes of treatment available and an evaluation of the technic to be applied, depending on the stage of the disease. It is concluded that all patients with operable tumors (Groups I and II—Portmann's classification) should receive preoperative irradiation followed 6 to 8 weeks later by radical mastectomy. The Group III cases (inoperable) should be treated by irradiation, but subsequent surgical measures should be used with extreme discretion. Local recurrences and distant metastases, especially those involving the skeleton, respond relatively well, at least temporarily, to radiation therapy.—A. C.

Cancer of the Uterus. Results of the Present Method of Radium Therapy as Influenced by Stage and Grade of the Lesion. Bowing, H. H., and Fricke, R. E. [Mayo Clinic, Rochester, Minn.] *Am. J. Roentgenol.*, **49**:487-493. 1943.

Survival rates of large series of patients treated at the Mayo Clinic for cancer of the cervix and of the uterine fundus are analyzed according to stage, and to grade of malignancy. It is concluded that the extent of the primary lesion is the most valuable prognostic factor. Broder's index of grade of malignant change is helpful when rather standardized methods of treatment are employed, but is less useful when individualized therapy is used.—E. H. Q.

Secondary Lymphosarcoma of the Stomach. Buschke, F., and Cantril, S. T. [Swedish Hosp., Seattle, Wash.] *Am. J. Roentgenol.*, **49**:450-454. 1943.

Attention is called to the possibility of confusing a single metastatic lymphosarcoma in the stomach with a primary lymphosarcoma or carcinoma of that organ. The prognosis in the 3 cases would be very different.—E. H. Q.

Contact Roentgen Therapy of Superficial Malignant Lesions about the Eye. Howe, W. E., and Camiel, M. R. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Arch. Ophth.*, **29**:224-230. 1943.

The technic of treating superficial malignant lesions about the eye with radiation therapy is described.—E. C. R.

Panhysterectomy Versus Irradiation for Early Cancer of the Uterine Cervix. JONES, H. W., JR., and JONES, GEORGEANNA E. S. [Johns Hopkins Univ. and Hosp., Baltimore, Md.] *J. A. M. A.*, **122**:930-932. 1943.

The authors conclude, after a study of 36 carefully selected patients with early carcinoma of the cervix treated by panhysterectomy, that this is an unsatisfactory method of therapy. Irradiation is the treatment of choice.—M. E. H.

The Betatron. KERST, D. W. [Univ. of Illinois, Urbana, Ill.] *Radiology*, **40**:115-119. 1943.

The betatron is a new apparatus for accelerating electrons, which is now in operation at the University of Illinois. The electrons are injected into a doughnut-shaped vacuum tube that is surrounded by an electromagnet producing an alternating magnetic field. The electrons are injected when the magnetic field is small and make circular orbits as the magnetic field increases in intensity. The electrons receive an acceleration with each turn, and when the magnetic field reaches its maximum intensity, the speed approaches that of light and corresponds to an energy ranging from 10 to 50 million volts according to the design and setting of the apparatus. With very careful shaping of the pole pieces, the stream of electrons is held within a circle and then directed toward a window at the time of maximum intensity of the magnetic field. The betatron may be looked on as a transformer, the secondary of which consists of these electrons, and the number of turns is the number of circular trips made by them. The electrons may be used as such, or they may hit a target located at the window and their energy be transformed into x-rays. If the electromagnetic field has 60 cycles per second, there are 60 streams of electrons giving, for practical purposes, a steady flow. An output of as great as 50 r per minute at 70 cm. distance has already been obtained. A 100-million volt betatron has been designed.—R. E. S.

Experimental Depth Dose for 5, 10, 15 and 20-Million-Volt X-rays. KOCH, H. W., KERST, D. W., and MORRISON, P. [Univ. of Illinois, Urbana, Ill.] *Radiology*, **40**: 120-127. 1943.

The betatron is not yet ready for practical use, but measurements indicate its possible applications in therapy. The primary electrons may be used as such, or their energy may be transformed into very penetrating x-rays that give the secondary electrons, the range of which is several centimeters. Original electrons used from the 20-million-volt betatron penetrate 10 cm. in the body and no farther. Maximum ionization should be obtained at 7 to 8 cm. If x-ray is used the maximum of ionization is several centimeters beneath the skin, and the depth of this maximum increases with the voltage, thus giving the possibility of elective depth of maximum ionization. This maximum is several times greater than the surface dose. There is a possibility of giving very high doses to a deeply located tumor without damaging the skin, by regulating the depth of maximum ionization according to the depth of the tumor. The depth dose curves were taken at 5, 10, 15, and 20-million-volt x-rays. One point must not be forgotten, *i.e.*, that the exit dose is greater than the entrance dose, and the skin could be damaged if this is not taken into consideration.—R. E. S.

Radiation Treatment of Cancer of the Cervix. MCCORMICK, N. A. [Metropolitan General Hosp., Windsor, Ontario, Canada] *Canad. M. A. J.*, **49**:178-184. 1943.

The treatment recommended is based on the technic developed at the Curie Foundation, Paris, and at the Memorial Hospital, New York. One hundred and thirty-five cases were studied, and the conclusions are based on 67 cases of primary carcinoma of the cervix, selected for analysis. It is stated that this type of cancer should be treated by radiotherapeutic methods without previous surgical intervention and with as little manipulative trauma as possible. The technic consists in intensive roentgen irradiation (60 or more treatments in the course of 35 to 40 days) followed by a short period of radium application, amounting to a total of from 5,500 to 6,000 mgm. hours. Fifty per cent of the patients are living normal lives 5 years after treatment. The results appear superior to those obtained by earlier methods.—A. C.

Further Experience with Pneumoperitoneum as an Aid in Pelvic Irradiation. SANTE, L. R. [St. Louis City Hosp., St. Louis, Mo.] *Radiology*, **40**:447-453. 1943.

In order to spare the intestinal tract and increase radiation to the pelvic tissues, Sante produces a pneumoperitoneum during the course of external irradiation for carcinoma of the cervix. One refill is usually necessary during the treatment. Radium is given by the usual technic. Too short a time has elapsed to permit an evaluation of results. In the discussion of this paper Dr. Stone points out that the depth dose is decreased by the introduction of air.—R. E. S.

Intravaginal Roentgen Irradiation of Carcinoma of the Cervix. WASSON, W. W. [Denver, Colo.] *Radiology*, **40**:454-457. 1943.

For intravaginal therapy Wasson uses cylinders of various sizes and checks positioning with the periscope. The cylinder is placed in 5 different positions in the vaginal canal in rotation to give uniform distribution of radiation. One hundred and forty or 200 kilovolts are used depending on the size of the patient. No information concerning results is available as yet.—R. E. S.

EYE

Bilateral Metastatic Carcinoma of Choroid. Report of a Case. MCBEAN, G. M. [Chicago, Ill.] *Arch. Ophth.*, **30**:776. 1943.

Report of a case.—E. C. R.

Reticulin Content and Prognosis in Malignant Melanoma of the Uvea. MCGREGOR, I. S., and HILL, J. [Glasgow, Scotland] *Arch. Ophth.*, **30**:291-297. 1943.

Forty-one specimens of malignant melanoma were examined histologically. It was found that the survival time of the patient cannot be gauged accurately from the histologic characteristics or reticulin content of the tumor.—E. C. R.

Retinoblastoma of Infants and Children. Its Significance as a Pediatric Problem. NICHAMIN, S. J. [Children's Hosp. of Michigan and Wayne Univ. Med. Sch., Detroit, Mich.] *Am. J. Dis. Child.*, **63**:945-953. 1942.

A case of retinoblastoma in a 3 year old child is reported, and a discussion relating to nomenclature, pathology, symptomatology, diagnosis, and treatment is presented.—C. J. M.

Primary Carcinoma of the Lacrimal Punctum. Streicher, C. J. [Canton, Ohio] *Ohio State M. J.*, **38**:240. 1942.

The common tumors of the eyelids are listed as chalazion, nevus, melanoma, warts, squamous and basal cell carcinoma, and xanthoma. Carcinoma of the lacrimal punctum is believed to be very rare. A small tumor, 4 × 3 mm., was removed from this region in a 50 year old male. The only symptom had been epiphora. Normal function of the lacrimal system was restored after resection and x-ray therapy.—E. E. S.

Female Genital Tract

The Brenner Tumour of the Ovary. Ayre, J. E., and Kearns, P. J. [Royal Victoria Hosp., Montreal, Canada] *Canad. M. A. J.*, **49**:404-407. 1943.

Diagnosis in all cases of Brenner tumor depends upon microscopic examination. The characteristic feature is the presence of epithelial cell nests surrounded by variable amounts of condensed fibromatous stroma. The paper includes the report of a case that was treated surgically.—A. C.

A Method of Obtaining Endometrial Smears for Study of Their Cellular Content. Cary, W. H. [Cornell Univ. Med. Coll., New York, N. Y.] *Am. J. Obst. & Gynec.*, **46**:422-424. 1943.

Description of the apparatus and technic developed for obtaining endometrial and endocervical secretions for study as described by Papanicolau and Marchetti (*Am. J. Obst. & Gynec.*, **46**:421. 1943).—A. K.

The Management of the Cervix in the Treatment of Fibromyoma of the Uterus. Corscaden, J. A. [New York, N. Y.] *New York State J. Med.*, **43**:829-835. 1943.

A general discussion, with 5 illustrative case histories.—J. L. M.

Carcinoma of the Vulva and Vagina in Infancy. Hoge, R. H., and Benn, V. A. [Medical Coll. of Virginia, Richmond, Va.] *Am. J. Obst. & Gynec.*, **46**:286-290. 1943.

This is a report of a case of carcinoma of the vulva in an infant, who is, perhaps the youngest recorded patient with this disease. Signs of the condition appeared when the child was 5 months of age; a visible external lesion was present at 16 months; microscopic diagnosis was made at 21 months, the lesion being recorded as a grade 4 adenocarcinoma. Radium and x-ray treatment were used, but death occurred when the child was 3 years old, after metastasis had developed in the lungs.—A. K.

Mesonephroma of the Ovary. Jensik, R. J., and Falls, F. H. [Univ. of Illinois, Coll. of Med., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **46**:810-816. 1943.

A case report. A malignant ovarian tumor classified as a mesonephroma is described. The term "mesonephroma" was applied by Schiller to certain types of ovarian tumors differentiated from the ordinary capillary cystic types. The tumor was believed to arise from a portion of the embryonic mesonephros incorporated in the ovary. The authors mention the arguments concerning the histogenesis of the tumor.—A. K.

Hypernephroma of the Ovary. Kannerstein, M., Brown, C. R., and Rosen, J. A. [Lincoln Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **46**:290-294. 1943.

A case is reported of ovarian tumor believed to be secondary to a renal hypernephroma removed surgically 11 years before. Ovarian metastases of hypernephromas are said to be rare, and this constitutes the third, or possibly the fourth, case on record.—A. K.

Theca Cell Tumor of the Ovary and Carcinoma of the Endometrium. Kirshbaum, J. D. [Cook County Hosp., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **46**:573-576. 1943.

A case report. Adenocarcinoma of the endometrium and uterine fibromyomas were found associated with a theca cell tumor of the ovary. Theca cell tumors elaborate an estrogenic hormone causing hyperplasia of the endometrium. Under continuous estrogenic stimulation malignant transformation of the hyperplastic endometrium is possible. The author feels that in this case there was a causal relationship between the ovarian tumor and the endometrial carcinoma.—A. K.

Superficial Noninvasive Intraepithelial Tumors of the Cervix. Knight, R. van D. [Sloane Hosp. for Women, New York, N. Y.] *Am. J. Obst. & Gynec.*, **46**:333-349. 1943.

Seventeen cases of superficial epithelioma of the cervix are reported. The author feels that non-invasive epithelial tumors of the cervix occur more frequently than is generally supposed; they develop more slowly and seem to be less malignant than the obvious epitheliomas. Treatment should be just as vigorous for the superficial, as for the infiltrative type.—A. K.

Bowen's Disease of the Vulva. Knight, R. van D. [Sloane Hosp. for Women, New York, N. Y.] *Am. J. Obst. & Gynec.*, **46**:514-524. 1943.

A report of 6 cases. The author feels that Bowen's disease is a specific entity, namely, a superficial, noninvasive, intraepithelial epithelioma characterized by chronicity, pruritis, and a distinctive gross and microscopic appearance. It is primarily a skin disease; when it involves mucosal surfaces it shows more malignant tendencies than it does otherwise. The treatment of choice is local wide excision.—A. K.

A Consideration of Certain Factors Pertaining to the Control of Carcinoma of the Cervix. Miller, N. F. [Univ. of Michigan Hosp., Ann Arbor, Mich.] *Am. J. Obst. & Gynec.*, **46**:625-634. 1943.

The subject of carcinoma of the cervix is briefly reviewed with the conclusions that the 5 year cure rate provides no cause for satisfaction with present methods of treatment. Either radiation therapy is not an adequate form of treatment or it is not being used to best advantage. So far the results of lay education concerning this type of cancer have been disappointing. Further education, especially with the use of films, is advocated.—A. K.

The Use of Endocervical and Endometrial Smears in the Diagnosis of Cancer and of Other Conditions of the Uterus. Papanicolaou, G. N., and Marchetti, A. A. [Cornell Univ. Med. Coll., and New York Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **46**:421-422. 1943.

Compared with the vaginal smear, the uterine smear shows a larger number and a greater variety of endometrial and cervical cells. Diagnosis of cancer of the fundus as well as of the cervix is thus greatly facilitated. In the uterine smear, certain cytological features are better shown; also endometrial cells can be obtained in the absence of bleeding, whereas in the vaginal smear endometrial cells are seen chiefly during the menstrual flow. The vaginal

smear has the advantage of simplicity and facility in routine application, whereas in the case of the endometrial smear there are many contraindications, especially those of infection or pregnancy.—A. K.

Fibroids in Pregnancy. Randall, J. H., and Odell, L. D. [University Hosp., Iowa City, Iowa] *Am. J. Obst. & Gynec.*, **46**:349-357. 1943.

A study of 17 cases forms the basis of the following conclusions: There is no hypertrophy of the smooth muscle fibers or hyperplasia of the connective tissue stroma within fibroids during pregnancy. Fifty per cent to 75% of fibroids show degenerative changes during this period, probably as a result of inadequate blood supply. Edema on the basis of severe degenerative changes could explain the enlargement of fibroids during pregnancy; such enlargement should be accompanied by symptoms. Any suspected enlargement of asymptomatic fibroids during pregnancy is only apparent.—A. K.

Experiences in the Treatment of Carcinoma of the Cervix Uteri. Scheffey, L. C. [Jefferson Med. Coll., Philadelphia, Pa.] *Radiology*, **40**:436-446. 1943.

The cases of carcinoma of the cervix treated at Jefferson Medical College Hospital from 1921 to 1937 were analyzed with respect to management, treatment, and end results. A total of 310 patients was seen, and 293 were treated. Ninety-eight per cent were traced in the follow-up study. The absolute salvage rate was 14.3%; the relative salvage rate, including patients who died after 5 years, was 23.8%. Carcinoma of the cervical stump was encountered in 5.1% of patients; of these, 50% survived 5 years or more. Only 1.6% had had previous irradiation for benign conditions. Present treatment consists of transvaginal x-ray therapy in conjunction with preliminary external radiation and the local use of radium.—R. E. S.

An Introduction to the History of Carcinoma of the Cervix Uteri. Skinner, E. H. [Kansas City, Mo.] *Radiology*, **40**:433-435. 1943.

In a historical review, the author traces briefly the development of treatment of carcinoma of the cervix from the earliest methods on record to the present day. He then suggests the possibility of a vaginal hysterectomy at the end of the child-bearing period as a prophylactic measure.—R. E. S.

Male Genital Tract

Subcapsular Orchidectomy in Advanced Prostatic Carcinoma. Burns, E., and Kittredge, W. E. [Tulane Univ. Sch. of Med., and the Ochsner Clinic, New Orleans, La.] *Surg. Clin. No. America*, **23**:1367-1376. 1943.

The rationale of orchidectomy in the treatment of carcinoma of the prostate is discussed, and the results of a series of 23 cases of subcapsular orchidectomy are reported. The technic of subcapsular orchidectomy is described and illustrated. There were no operative deaths. Undesirable results include complete loss in sexual power in all cases and hot flashes in a few. The hot flashes may sometimes be relieved by the administration of stilbesterol. —J. L. M.

Carcinoma of the Prostate. A Study of the Percentage of Cases Suitable for the Radical Operation. Colston, J. A. C. [Johns Hopkins Hosp., Baltimore, Md.] *J. A. M. A.*, **122**:781-784. 1943.

For the 5 year period from 1937 to 1942 the diagnosis of carcinoma of the prostate was made in 358 cases. Seventy-three patients (20.2%) submitted to radical operations. Among them, there were 4 hospital deaths, a mortality of 5.5%. Of 43 patients for whom the prognosis was good, 41 are living and well without evidence of recurrence or metastasis. Of 26 patients for whom the prognosis was poor, 8 are living and well at intervals varying from 3 months to 5 years.—M. E. H.

The Treatment of Benign Prostatic Hyperplasia in Relation to Prostatic Carcinoma. Greene, L. F., and Thompson, G. J. [Mayo Clinic, Rochester, Minn.] *J. A. M. A.*, **122**:790-793. 1943.

Surgical treatment of benign prostatic hyperplasia is undertaken in an effort to relieve the symptoms of urinary obstruction. The performance of suprapubic or simple perineal prostatectomy does not preclude the subsequent development of prostatic carcinoma or recurrent prostatic hyperplasia. Radical prostatectomy, in which the entire prostate gland, a portion of the urethra, a cuff of the bladder, both seminal vesicles, and 5 cm. of each vas deferens are removed, is employed exclusively in cases of prostatic carcinoma and is applied with hope of cure in only about 3% of cases. Although by suprapubic or simple perineal prostatectomy an undetected carcinoma within an adenoma may be removed, a happy outcome is infrequent. More frequently the lesion has spread to the perineal lymphatic vessels, and then cure by any of the 3 methods outlined for the relief of symptoms due to prostatic hyperplasia is impossible.—M. E. H.

Histological Changes in Carcinoma of Prostate Following Resection and the Use of Stilbœstrol. Hall, E. R. [Vancouver, Canada] *Canad. M. A. J.*, **48**:441-442. 1943.

The successful removal of a carcinoma of the prostate in a 62 year old patient was followed by persistent discomfort in the region of the rectum and by a pronounced feeling of fatigue. Fifteen months after operation stilbestrol therapy (5 mgm. daily) was instituted. After about a year of treatment (total amount of stilbestrol, 1,000 mgm.) the patient had gained weight, his ability to work had been restored, and the local symptoms had disappeared. The prostate was smaller and more regular in shape, giving the impression of a normal gland. A biopsy performed at the time of the report showed that carcinoma cells were still present, but the general microscopic picture was suggestive of involution of the neoplastic elements.—A. C.

The Diagnosis and Treatment of Early Carcinoma of the Prostate. Henline, R. B. [New York Hosp., New York, N. Y.] *J. A. M. A.*, **122**:785-789. 1943.

Cancer of the prostate has been found in approximately 1 out of every 7 men more than 50 years of age. There are no symptoms of early prostatic carcinoma. A careful digital palpation of the prostate should be part of the physical examination of every man who has reached this age period.—M. E. H.

Endocrine Control of Prostatic Cancer. Huggins, C. [Univ. of Chicago, Chicago, Ill.] *Science*, **97**:541-544. 1943.

This paper reviews the endocrine background and the present state of knowledge concerning the control of prostatic cancer by castration or administration of estrogen.

The normal prostate is under the control of two types of hormones. Androgens bring about an increase of size of the gland, and the initiation and maintenance of the function of prostatic epithelium. Estrogens have the opposite effect through their capacity to "neutralize" the activity of androgens with respect to the prostate.

Prostatic cancer retains some of the properties of normal prostatic epithelium. Particularly, it produces acid phosphatase in such amounts that the increase of this enzyme in the blood may be used as a diagnostic test in advanced cases of the disease. When the bones are invaded there is an increase also in alkaline phosphatase resulting from increased osteoblastic activity. Furthermore, prostatic cancer frequently retains the normal property of reacting to androgens and estrogens. Reduction of androgens by castration or estrogen administration is followed by a sharp fall toward normal values of acid phosphatase in the blood. Alkaline phosphatase increases slowly for several weeks, apparently as a result of healing of bony lesions, after which there is a decrease of this enzyme. Along with these changes in the phosphatases there is a relief of pain, an improvement in appetite, and often a pronounced decrease in the size of the tumor and its metastases.

The results of endocrine treatment of prostatic cancer fall into three groups. Less than 5% of patients receive little or no benefit. The remaining two groups, larger and equal in number, show respectively an improvement that is pronounced but unsustained (less than 18 months), or a pronounced and prolonged regression of the disease. The author suggests that the failure cases may be due to secretion of androgens in organs other than the testes, for example the adrenal cortex, or to differences in the nature of the original tumor.—R. B.

Castration for Carcinoma of the Prostate. A Report on Fifteen Treated Cases. Smith, E., and MacLean, J. T. [Royal Victoria Hosp., Montreal, Canada] *Canad. M. A. J.*, **49**:387-392. 1943.

At the time of the report 1 year had elapsed since operation in 1 case, 6 months in 7 cases, and less than 6 months in 7 others. The age of the patients ranged from 58 to 88 years. All of them had far advanced carcinoma of the prostate on rectal examination, the diagnosis being confirmed by biopsy in 12. In the majority of cases there was complete relief of pain within 48 hours, a pronounced improvement in appetite, a gain in weight (15 to 30 pounds), and an increase in the red blood cell count; in 2 patients with prostatic obstruction the symptoms disappeared entirely. Roentgenograms failed to show regression of the metastatic lesions, and in 1 case, there was definite progress of the lesion. Thus, from these early results it can not be stated that castration is a cure for carcinoma of the prostate. An increase in serum acid phosphatase is considered to be pathognomonic of metastasizing prostatic carcinoma.—A. C.

Urinary System—Male and Female

Sarcoma of the Bladder in an Infant. Bugbee, H. G., and Dargeon, H. W. [St. Luke's Hosp., New York, N. Y.] *J. Pediat.*, **19**:656-661. 1941.

Report of a case of sarcoma of the bladder in a 9 month old boy. The tumor gave the appearance of an enlarged prostate; it was located at the vesical neck and extended into the urethra. The disease was complicated by infection of the bladder and kidneys. Attempts at treatment by irradiation were unsuccessful.—A. C.

Neurofibromatosis of the Bladder in a Nine-Year-Old Boy. Chalkley, T. S., and Bruce, J. W. [Louisville City Hosp., Louisville, Ky.] *J. Pediat.*, **20**:632-636. 1942.

Diagnosis was made by means of exploratory laparotomy and biopsy. The patient and 4 sibs had multiple areas of pigmentation on the skin but no evidence of skin tumors. The mother, in addition to pigmented areas, had multiple neurofibromas of the skin.—A. C.

Cancerous Mixed Tumor of the Urinary Bladder. Hirsch, E. F., and Gasser, G. W. [St. Luke's Hospital., Chicago, Ill.] *Arch. Path.*, **37**:24-26. 1944.

A pedunculated growth of the trigone in a man of 83 contained islets of cartilage and other mesoblastic tissues. The epithelial components were limited to a thin surface layer of squamous epithelium and to small masses of similar cells nearby that seemed to be extensions into crevices. Two figures illustrate the various types of neoplastic cells. The literature yields but few reports of similar cases.—J. G. K.

Oral Cavity and Upper Respiratory Tract

A Case of Carcinoma of the Naso-Pharynx. de Boissiere, V. [Homœopathic Hosp., Montreal, Canada] *Canad. M. A. J.*, **49**:176-177. 1943.

A case report.—A. C.

Cancer of the Larynx. (With Observations on 103 Cases.) Campbell, A. A. [Toronto, Canada] *Canad. M. A. J.*, **49**:509-512. 1943.

In the series reported, males were affected almost 6 times as frequently as females. Many of the males were heavy smokers. Except for 1 sarcoma, all the tumors were epidermoid carcinoma. Constant and persistent hoarseness was the most common symptom; in 6 cases, the first symptom was the presence of enlarged glands in the neck. Of 103 cases, only 9 were considered suitable for laryngo-fissure, and in only 4 of these did surgical treatment succeed. Three of 5 patients who submitted to laryngotomy may be considered cured, 5, 5, and 16 years respectively, after operation.—A. C.

Epidermoid Carcinoma in the First Decade of Life. Report of a Case. Carey, R. M. [Univ. of Pittsburgh, and Children's Hosp., Pittsburgh, Pa.] *J. Pediat.*, **20**:496-498. 1942.

The tumor, an epidermoid carcinoma associated with mucus, developed on the inner surface of the cheek when the infant was 3 months old. The growth was removed under anesthesia with no apparent recurrence 10 months after operation.—A. C.

Carcinoma of the Antrum. Thompson, H. E. [Dubuque, Minn.] *J. Iowa M. Soc.*, **33**:550-553. 1943.

Report of a case, including a description of the treatment by surgery, radium, and x-ray. There was no recurrence of the tumor 6 years after operation. A general discussion of the subject is based on 12 instances of malignant epithelial tumors of the nasopharynx or antrum treated at this clinic during the past 14 years.—A. C.

Intrathoracic Tumors—Lungs—Pleura

Bronchiogenic Carcinoma. With Special Reference to the Management of the Bronchial Stump in Total Pneumonectomy. Adams, H. D. [Lahey Clinic, Boston, Mass.] *Surg. Clin. No. America,* **23**:881-886. 1943.

A technic for the management of the bronchial stump in total pneumonectomy is described and illustrated. Five case reports are presented.—J. L. M.

Primary Bronchiogenic Carcinoma. Report of a Five-Year Surgical Cure. Kinsella, T. J. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Minnesota Med.,* **26**: 90-96. 1943.

Primary bronchiogenic carcinoma is discussed. The case is reported of a man who submitted to total left pneumonectomy and is in good condition 5 years after operation.—J. L. M.

Carcinoma of the Lung. Moersch, H. J., and Tinney, W. S. [Mayo Clinic, Rochester, Minn.] *Minnesota Med.,* **26**: 1046-1051. 1943.

From 1925 to 1942, inclusive, the clinical diagnosis of bronchiogenic carcinoma has been made in 948 cases at the Mayo Clinic. An analysis is presented of 448 cases in which the diagnosis was confirmed by microscopic examination.

A follow-up study was made in 315 cases. The average duration of life after diagnosis was made was 6 months. The prognosis was the same in both adenocarcinoma and squamous cell carcinoma, regardless of the grade of the tumor. The average duration of illness from the first symptom until death was 14½ months. Since it required an average of 8½ months after onset of symptoms to make the diagnosis, the patients had obviously lived more than half their expectancy before there was any opportunity to consider surgical exploration. Delay is undoubtedly the most important cause of the poor prognosis of bronchiogenic carcinoma.—J. L. M.

Ganglioneuroma of the Mediastinum. Skinner, G. F., Branch, A., and Allen, I. [St. John Tuberculosis Hosp., and the Provincial Bureau of Labs., St. John, N. B.] *Canad. M. A. J.,* **49**:397-399. 1943.

The tumor was found incidentally on routine x-ray examination of the chest, in a 10 year old girl. Thoracotomy and removal of the tumor were successful.—A. C.

Bronchiogenic Carcinoma of Seven Years' Duration in an 11-Year-Old Boy. Wasch, M. G., Lederer, M., and Epstein, B. S. [Jewish Hosp., Brooklyn, N. Y.] *J. Pediat.,* **17**:521-528. 1940.

The tumor was diagnosed, with the aid of biopsy, as a bronchiogenic adenocarcinoma when the boy was 11 years old. The patient survived 7 years. While under observation, he developed an eosinophilic adenoma of the pituitary, with resulting symptoms of gigantism. The lung neoplasm responded surprisingly well to radiation treatment, especially to radium therapy. Numerous metastases in the myocardium and heart failure were the immediate causes of death.—A. C.

Gastrointestinal Tract

Carcinoma of the Colon. Allen, A. W. [Boston, Mass.] *Surgery,* **14**:350-365. 1943.

A general discussion. It is suggested that preliminary ileotransverse colostomy with aseptic suture be used for lesions of the right colon and proximal third of the transverse colon, and preliminary tube cecostomy for lesions of the remaining colon. Resection with immediate anastamosis is the method of choice for the second stage. Delayed closure of the abdominal wound by Coller's technic is recommended.—W. A. B.

Primary Sarcoma of the Duodenum. Bisgard, J. D., and Cochran, R. M. [Univ. of Nebraska Coll. of Med., Omaha, Nebr.] *Am. J. Surg.,* **61**:425-429. 1943.

Report of a case treated by resection with removal of the head of the pancreas by the one stage Whipple operation.—W. A. B.

Carcinoma of the Colon and Rectum. A Report of 503 Patients Treated at the Lahey Clinic 1938-1941, Inclusive. Cattell, R. B. [Boston, Mass.] *Surgery,* **14**: 378-386. 1943.

The histories of 331 patients treated during 1938, 1939, and 1940 are reviewed; 191 patients were males. Carcinoma of the rectum, including the rectosigmoid, accounted for 62.15% of the cases, and carcinoma in the colon or sigmoid, for 37.9%. The duration of symptoms was less than 1 year in 60.7% and less than 6 months in 38.4%. In 38.8% of 280 resections there was no gross or microscopic evidence of metastases, in 40% there was invasion of regional lymph nodes, and in 9.6% hepatic metastases were present.

Of 503 patients seen from 1938 to 1941 inclusive, resection was done on 420 (83.5%), with 45 operative deaths (10.7%). Among 168 patients who had a one-stage abdominoperineal resection there were 11 deaths (8.5%). There were 12 deaths in 87 patients who had a two-stage abdominoperineal resection; 3 deaths among 15 patients submitting to perineal resection; and 15 deaths among 133 patients with Mikulicz resections.

A summary of the end results of 162 resections done during the years 1932 to 1936 inclusive showed that 75 patients (46.3%) survived from 5 to 9 years. Twenty-two died postoperatively, and 65 died of recurrence.—W. A. B.

The Management of Polyps Occurring in the Rectum and Colon. David, V. C. [Chicago, Ill.] *Surgery,* **14**:387-394. 1943.

The technic of removal is described, and emphasis is laid on the danger of malignant degeneration of polyps of the colon and rectum.—W. A. B.

Anterior Resection of the Rectosigmoid and Upper Rectum with Re-establishment of Continuity. Fallis, L. S. [Henry Ford Hosp., Detroit, Mich.] *Surgery,* **14**:397-402. 1943.

The technic is described.—W. A. B.

Significance of Schwannomas as a Factor in Obscure Cases of Appendicitis. Laird, W. R., and Nolan, L. E. [Memorial Hosp., Montgomery, W. Va.] *Am. J. Surg.,* **61**:418-420. 1943.

Thirteen of 14 patients with right lower quadrant pain were relieved by appendectomy. In all 14 cases, neuroma (Schwannoma) was found.—W. A. B.

Lipomas of the Gastrointestinal Tract. Schottenfeld, L. E. [Jewish Hosp., Brooklyn, N. Y.] *Surgery,* **14**: 47-72. 1943.

Approximately 275 lipomas of the gastrointestinal tract are on record. Six more are reported. They occur most

frequently as single tumors, and more than half (56%) are in the small intestine. About 90% arise in the submucosa; most of the subserous lipomas occur in the large intestine where they originate from the appendices epiploicae. The symptoms are those of intestinal obstruction, produced by the tumor itself or by intussusception. Preoperative diagnosis is rarely if ever made.—W. A. B.

Blood Vessels

Hemangiopericytoma. A Vascular Tumor Featuring Zimmermann's Pericytes. Stout, A. P., and Murray, M. R. [Coll. of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Ann. Surg.*, **116**:26-33. 1942.

Nine cases are described of a vascular tumor characterized by endothelial tubes and sprouts with a surrounding sheath of rounded and sometimes elongated cells. These are presumed to be derived from capillary pericytes (of Zimmermann), which are contractile cells with long processes that encircle capillaries and serve to regulate the caliber of their lumens. In one patient the tumor was malignant and in another, probably malignant.—W. A. B.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Leukemia Resembling Chloroma. Brakeley, E. [Mountainside Hosp., Montclair, N. J.] *Am. J. Dis. Child.*, **64**:689-696. 1942.

Report of a case in a 2½ year old boy.—C. J. M.

Chronic Myelogenous Leucemia in Early Infancy. A Case Report. Poncher, H. G., Weir, H. F., and Limarzi, L. R. [Univ. of Illinois Coll. of Med., Chicago, Ill.] *J. Pediat.*, **21**:73-79. 1942.

Report of a case of chronic myeloid leukemia in a 6 week old infant. The child died of bilateral bronchopneumonia. Treatment of the leukemic condition by roentgen irradiation had been ineffective. From a review of the cases available in the literature, this appears to be the earliest age at which this disease has been recognized.—A. C.

Hodgkin's Disease in an Infant. Report of a Case with a Peculiar Peripheral Blood Picture. Schwind, J. L., and Hyde, G. M. [Albany Med. Coll., Union Univ., Albany, N. Y.] *J. Pediat.*, **21**:238-245. 1942.

Ths case of Hodgkin's disease was apparently not congenital since, on repeated examination, the mother and a child born subsequently showed no evidence of the disease. The patient, a girl, had been found normal upon examination 10 days after birth, and was 4 months old when the first symptoms were noticed. The illness gave the characteristic picture of Hodgkin's disease of the abdominal type with a very rapid course, causing death within 2 months, with extensive lesions in practically every organ. The course of the disease was marked by intermittent fever. The peripheral blood contained an abnormal type of lymphocyte, which might have led to an erroneous diagnosis of lymphocytic leukemia.—A. C.

Xanthoma or Chloroma. Follow-up Data on Case of "Chloroma" Reported in 1930. Washburn, A. H., and Christie, A. U. [Sch. of Med., Univ of Colorado, Denver, Colo. and the Univ. of California Med. Sch., San Francisco, Calif.] *Am. J. Dis. Child.*, **63**:335-345. 1942.

Further report of a case that has been followed for 15 years. The diagnosis remains in doubt.—C. J. M.

Miscellaneous

A Hard Rubber Plaque for Anchoring and Facilitating the Removal of Radium Applications to the Body of the Uterus. Anspach, B. M. [Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **41**:341. 1941.

A description and illustration of the device.—A. K.

Teratoma of the Pineal Body. Gerstley, J. R., Kasanin, J., and Lowenhaupt, E. [Sarah Morris Hosp. for Children and Michael Reese Hosp., Chicago, Ill.] *J. Pediat.*, **17**:512-520. 1940.

The paper describes a case of teratoma of the pineal body in an 11 year old boy, the 18th tumor of this type to be reported. A spongioblastoma multiforme of unilateral development within the tumor, invaded the surrounding structures widely, infiltrating the hypothalamus.

Symptoms of pineal tumors are of two types: (a) Mechanical signs of increased intracranial pressure, which may be intermittent. (b) Endocrine disturbances that may produce the striking picture of macrogenitosomia praecox. The child showed pubertas praecox, initial symptoms of pituitary cachexia, and finally, signs characteristic of involvement of the quadrigeminate plate and the hypothalamus. Neurological symptoms were late. The patient had all the outward manifestations of depression, without being aware of such emotion. The course of the disease was marked by increased apathy, anorexia, persistent vomiting of all solid food, and progressive weight loss till death.—A. C.

Multiple Tissue Carrier for Histologic Techniques. Kessel, A. M. [National Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **4**:361-362. 1944.

Branchiogenous Carcinoma in a Child. Parkinson, S. N. [Oakland, Calif.] *Am. J. Dis. Child.*, **61**:1272-1274. 1941.

Report of a case in a boy of 7.—C. J. M.

Retroperitoneal Tumours in Children. Pilcher, F. [Calgary Associate Clin., Calgary, Canada] *Canad. M. A. J.*, **48**:505-510. 1943.

The paper includes a report of a case of Wilms' tumor in a 5 year old girl. The tumor was removed surgically, and extensive x-ray therapy was used both before and after operation. Death occurred 10 months later, 2 months after metastasis had appeared in the chest. Although used in this case, it is concluded that preoperative x-ray therapy should be avoided. It is felt that any delay in the surgical removal of the tumor and reactive changes brought about in the growth by radiations may favor the dissemination of tumor cells. Other retroperitoneal tumors of children are discussed.—A. C.

Book Reviews

SELECTED PAPERS FROM THE ROYAL CANCER HOSPITAL (FREE) AND THE CHESTER BEATTY RESEARCH INSTITUTE. Volume II. London. 1939-1940.

This volume contains the following papers, conveniently arranged according to subject.

JOLL, C. A. Trauma and Cancer. *West London M. J.*, **45**:58. 1940.

JOLL, C. A. Cancer of the Stomach. Med. Press & Circular, **204**:335. 1940.

ABEL, A. L. Experiences in Cancer of the Rectum. Arch. ital. di chir., **50**:1-8. 1938.

MAYNEORD, W. V. An Optical Device for the Accurate Alignment of an X-Ray Beam. *Brit. J. Radiol.*, **12**: 257. 1939.

ADAMS, S. B. A Simple Apparatus for the Rapid Positioning of Certain Deep Therapy Cases. *Brit. J. Radiol.*, **12**:259. 1939.

MAYNEORD, W. V. An Electric Adding Machine and Its Uses in Radiation Therapy. *Brit. J. Radiol.*, **12**:260. 1939.

MAYNEORD, W. V. A Dose Contour Projector and Its Application to Three-Dimensional Radiation Distributions. *Brit. J. Radiol.*, **12**:262. 1939.

HONEYBURNE, J., LAMERTON, L. F., SMITHERS, D. W., and MAYNEORD, W. V. Symposium on Three-Dimensional Radiation Distributions. *Brit. J. Radiol.*, **12**:269. 1939.

LAMERTON, L. F. A Physical Investigation of the Radiation from a Low-Voltage X-Ray Tube (Cautery Technique). *Brit. J. Radiol.*, **13**:136. 1940.

MAYNEORD, W. V. Total Energy Absorption in Biological Objects. Nature, **145**:972. 1940.

MAYNEORD, W. V. Energy Absorption. *Brit. J. Radiol.*, **13**:235. 1940.

LEDERMAN, M. Radium Therapy. *Post-Grad. M. J.*, **16**: 309. 1940.

LEDERMAN, M. Radium Treatment of Cancer of the Penis. *Brit. J. Radiol.*, **13**:393. 1940.

SMITHERS, D. W. The Treatment of Carcinoma of the Lip. *Post-Grad. M. J.*, **15**:376. 1939.

SMITHERS, D. W. The X-Ray Treatment of Malignant Tumours in the Region of the Eyes. *Brit. J. Ophth.*, **24**:105. 1940.

BADGER, G. M., and COOK, J. W. The Synthesis of Growth-Inhibitory Polycyclic Compounds. Part I. *J. Chem. Soc. London*, p. 802. May, 1939.

BADGER, G. M., and COOK, J. W. The Synthesis of Growth-Inhibitory Polycyclic Compounds. Part II. *J. Chem. Soc. London*, p. 409. April, 1940.

BADGER, G. M., COOK, J. W., HEWETT, C. L., KENNAWAY, E. L., KENNAWAY, N. M., MARTIN, R. H., and ROBINSON, A. M. The Production of Cancer by Pure Hydrocarbons. V. *Proc. Roy. Soc. s. B.*, **129**:439. 1940. (Abst. in *Cancer Research*, **1**:166. 1941)

BOYLAND, E., and BOYLAND, M. E. Studies in Tissue Metabolism. XII. The Action of Colchicine on Transplanted, Induced and Spontaneous Mouse Tumours. *Biochem. J.*, **34**:280. 1940. (Abst. in *Am. J. Cancer*, **39**:266. 1940)

BOYLAND, E. Experiments on the Chemotherapy of Cancer. IV. Further Experiments with Aldehydes and Their Derivatives. *Biochem. J.*, **34**:1196. 1940. (Abst. in *Cancer Research*, **1**:81. 1941)

BURROWS, H. Spontaneous Uterine and Mammary Tumours in the Rabbit. *J. Path. & Bact.*, **51**:385. 1940.

CHIBNALL, A. C., REES, M. W., WILLIAMS, E. F., and BOYLAND, E. The Glutamic Acid of Normal and Malignant Tissue Proteins. *Biochem. J.*, **34**:285. 1940. (Abst. in *Am. J. Cancer*, **39**:266. 1940)

COOK, J. W., and KENNAWAY, E. L. Chemical Compounds as Carcinogenic Agents. Second Supplementary Report: Literature of 1938 and 1939. *Am. J. Cancer*, **39**:381. 1940. (Abst. in *Cancer Research*, **1**:75. 1941)

COOK, J. W., ROBINSON, A. M., and ROE, E. M. F. Polycyclic Aromatic Hydrocarbons. Part XIX. *J. Chem. Soc. London*, p. 266. February, 1939.

COOK, J. W., and DE WORMS, C. G. M. Polycyclic Aromatic Hydrocarbons. Part XX. *J. Chem. Soc. London*, p. 268. February, 1939.

BADGER, G. M., COOK, J. W., and GOULDEN, F. Polycyclic Aromatic Hydrocarbons. Part XXI. *J. Chem. Soc. London*, p. 16. January, 1940.

HEWETT, C. L. Polycyclic Aromatic Hydrocarbons. Part XXII. *J. Chem. Soc. London*, p. 293. March, 1940.

COOK, J. W., and ROBINSON, A. M. Polycyclic Aromatic Hydrocarbons. Part XXIII. *J. Chem. Soc. London*, p. 303. March, 1940.

COOK, J. W., and MARTIN, R. H. Polycyclic Aromatic Hydrocarbons. Part XXIV. *J. Chem. Soc. London*, p. 1125. August, 1940. (Abst. in *Am. J. Cancer*, **40**:409. 1940)

EVERETT, J. L., and HEWETT, C. L. Polycyclic Aromatic Hydrocarbons. Part XXV. 1- and 2-Alkyl Derivatives of 3:4-Benzphenanthrene. *J. Chem. Soc. London*, p. 1159. August, 1940. (Abst. in *Am. J. Cancer*, **40**:409. 1940)

HEWETT, C. L., and MARTIN, R. H. Polycyclic Aromatic Hydrocarbons. Part XXVI. 1:2:3:4-Tetramethylphenanthrene. *J. Chem. Soc. London*, p. 1396. October, 1940.

COOK, J. W., HEWETT, C. L., KENNAWAY, E. L., and KENNAWAY, N. M. Effects Produced in the Livers of Mice by Azonaphthalenes and Related Compounds. *Am. J. Cancer*, **40**:62. 1940. (Abst. in *Cancer Research*, **1**:167, 1941)

HIEGER, I. The Examination of Human Tissue for Carcinogenic Factors. *Am. J. Cancer*, **39**:496. 1940.

IBALL, J. Oxidation-Reduction Potentials of Quinones Derived from Carcinogenic Hydrocarbons. *Am. J. Cancer*, **38**:372. 1940.

In the first paper, Joll can cite no reliable evidence, either experimental or clinical, that a single trauma can produce in its train, early or late, a malignant tumor. The evidence that injury can aggravate an existing malignant growth is very unsatisfactory, unless the injury be of such nature that an open wound is inflicted and

pathogenic microorganisms introduced from without into the tumor. There is urgent need, says the author, to bring our teaching of medical students and practitioners on this subject into line with the progress of modern cancer research, and this should eventually give rise to a necessary reform in the attitude of the Law and of Medicine to traumatic cancer.

The same writer, in his second paper, pleads again for the early diagnosis of carcinoma of the stomach. In a properly organized gastric clinic at least one-third of all the cases of gastric cancer dealt with should prove resectable, and of those patients who survive the operation at least one-third should be alive at the end of 3 years, one-quarter at the end of 5 years, and one-fifth at the end of 10 years.

This group of 3 clinical articles concludes with Abel's description of the results following abdomino-perineal excision for cancer of the rectum. Among 150 traced patients, 104 (69.3 per cent) were still well at the end of 5 years, and the author contends that this operation, when carefully planned and carefully executed, carries with it a lower mortality than any other radical method of attack, and has a much higher cure rate than any other form of treatment.

The 8 papers that follow are too severely technical for satisfactory condensation, but their contents are evident from their titles and those interested will wish in any case to consult them in their original form.

In a group of 4 articles on radiotherapy Lederman, and Smithers, discuss systemic effects of irradiation that often interfere with the course of treatment; the results of various technics for the treatment of carcinoma of the penis (53.8 per cent of 5-year and 61.9 per cent of 3-year cures); the favorable response of primary carcinoma of the lip to short-distance, low-voltage x-ray therapy; and the effect of x-rays on the eyes and the radiation treatment of growths in their vicinity.

The 19 papers remaining deal with experimental cancer research. Eleven of these are concerned with the synthesis and properties of polycyclic compounds, and will interest all chemists who have special knowledge of these substances. Three deal with carcinogenesis, of which one is a reprint of Cook and Kennaway's invaluable second supplementary report on the carcinogens. In another Badger and a group of associates describe the testing of a large number of hydrocarbons for carcinogenicity. Here the 3,4-benzphenanthrene compounds proved to be particularly interesting for they seemed to be more active than the benzanthrenes in the production of multiple tumors, cholangioma, and neoplasms of the lung, and the 1- and 2-substituted compounds were more active in regard to tumors of the stomach. In the third Cook, Hewett, and the Kennaways offer an account of the carcinogenic activity of the azonaphthalenes, in which they find a high degree of specificity. 2,2′-Azonaphthalene and its reduction product, 2,2′-diamino-1,1′-dinaphthyl, induced new growths in the liver; 1,1′-azonaphthalene had very little of this action; and 1,2′-azonaphthalene appeared to be inactive in this respect.

Two papers are devoted to chemotherapeutics. E. and M. E. Boyland found that the injection of large doses of colchicine injected into tumor-bearing mice elicited results similar to those produced by bacterial filtrates. Spontaneous neoplasms were much less affected than grafted tumors, and somewhat less than induced tumors, which occupied an intermediate position.

In another series of chemotherapeutic experiments E. Boyland sought to express quantitatively and statistically the effect of substances that inhibit tumor growth. Heptaldehyde exerted some inhibitory action on the growth of spontaneous carcinomas and transplanted sarcomas in mice, but a similar result was achieved with dicarboxylic acids (particularly malonic acid), which are possible metabolic products of heptaldehyde. Citral was more effective than heptaldehyde, but the monocarboxylic and dicarboxylic acids derived from citral had but little effect.

Hieger, finally, contributes a paper on the production of tumors in mice with extracts of livers from cancer and noncancer patients; Chibnall and his group record their failure to confirm the statement of Kögl and Erxleben that partial racemization is a characteristic of the proteins of malignant tissues; and Burrows describes the occurrence of uterine adenocarcinomas in 15 of 25 rabbits that had been under observation for more than 900 days. Three of these animals also had intracystic papillomas of the mammary gland that may have been malignant, and the author advances arguments for and against the possibility that the growths in both organs may have been brought about by ovarian hormones.

WM. H. WOGLOM

THE BIOCHEMISTRY OF MALIGNANT TUMORS. Kurt Stern and Robert Willheim. Reference Press, Brooklyn, N. Y. 1943. ix + 951 pages. Price $12.00.

The chemical and biochemical approach has been more and more utilized in the last 20 to 30 years in an attempt to broaden our understanding of the problem of the malignancy of the cell. Though this approach has far from solved the problem of cancer, it has tremendously increased our knowledge and has made fundamental contributions to cellular physiology. The research of Warburg and his collaborators on the gycolysis of tumors and the brilliant work of Cook, Kennaway, and their associates on the carcinogenic hydrocarbons illustrate most emphatically the validity of this approach.

The authors of the treatise under review summarize and interpret most of the chemical and biochemical work that has been done in the field of tumors. Though much of the data is of a controversial nature, and other data are difficult to evaluate, this accumulated body of knowledge, which can be presented only in brief form within the confines of a book, is developed in a critical, cautious, and exhaustive manner.

The subject matter will best indicate the scope of the book: inorganic, organic, and physical chemistry; enzymes; nutrition and vitamins; metabolism; endocrine glands and their hormones; immunology; the biochemical aspects of tumor origin and tumor growth; and chemical and biological tumor diagnosis.

DAVID SHEMIN.